

ABSTRACT SYMPOSIUM

Fibrinogen

AS002

Transcriptome analysis reveals HNF4A as a miR-29C target linked to reduced fibrinogen expressionLukowski SW^{1,2}, Fish RJ^{1,2} and Neerman-Arbez M^{1,2}¹Genetic Medicine and Development, University of Geneva;²Institute of Genetics and Genomics in Geneva (IGE3), Geneva, Switzerland

Background: The fibrinogen genes encode the three polypeptides of circulating soluble fibrinogen. Fibrinogen expression is controlled by transcription factors that interact with regulatory elements in the fibrinogen gene cluster. Fibrinogen mRNAs are sensitive to post-transcriptional regulation by miRNAs. In particular, overexpression of the miR-29 family (hsa-miR-29abc) leads to down-regulation of the three major fibrinogen transcripts but not as a consequence of a miR-29-fibrinogen mRNA interaction. We hypothesized that this fine-tuning of fibrinogen production is mediated via an upstream miR-29 target that could regulate all three fibrinogen chains.

Aims: This study aimed to uncover the regulatory pathway involved in miR-29-mediated control of fibrinogen expression.

Methods: Transcriptome analysis by RNA-seq was performed on fibrinogen-expressing HepG2 cells, with and without miR29c overexpression. MiR-29c-resistant mRNAs for candidate fibrinogen regulatory proteins were co-expressed with miR-29c to assess their potential to rescue fibrinogen expression.

Results: From over 1700 down-regulated differentially expressed genes, our analysis confirmed the repression of fibrinogen transcripts by miR-29c and identified a number of potential regulators of fibrinogen expression. These included the transcription factors HNF4A, RXRA and MAZ that are all predicted miR-29c target mRNAs. Overexpression of a miR-29c-resistant HNF4A mRNA reversed the miR-29c-mediated inhibition of the three fibrinogen genes, implicating HNF4A as a critical fibrinogen regulator that is sensitive to post-transcriptional regulation.

Conclusion: We identified HNF4A as a target for miR-29c and candidate fibrinogen regulator. Functional complementation of miR-29c-affected fibrinogen expression was attained by increased HNF4A expression. This provides a clear mechanism to the indirect effect of miR-29 on fibrinogen expression and identifies HNF4A as a master fibrinogen regulator.

Disclosure of Interest: None declared.

AS003

Characterisation of cellular fibrinogen phosphorylation and its functional implications in clot formation

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Background: Human fibrinogen is secreted from hepatocytes in its phosphorylated form, with 25% of circulating fibrinogen phosphorylated exclusively at A α chain Ser3 and Ser345. Phosphorylation of fibrinogen is elevated in acute phase conditions, venous thrombosis

and ovarian cancer, but little is known about the regulation and effects of this modification.

Aims: The aims of this study were to (i) determine the effects of acute phase mediator interleukin-6 (IL-6) on the synthesis of phosphorylated fibrinogen (p-fibrinogen), (ii) identify the kinase responsible, and (iii) examine the effects of fibrinogen phosphorylation on clot structure.

Methods: HepG2 cells were cultured $\pm$ IL-6 and the phosphate content of secreted fibrinogen (1 μ g) was analysed by western blotting with an anti-phosphoserine antibody, while changes in kinase expression were examined by real-time PCR. The effects of fibrinogen phosphorylation on clot structure were determined by scanning electron microscopy (SEM) and turbidimetric assays with purified enriched p-fibrinogen.

Results: A 3.1-fold increase in p-fibrinogen was observed in the presence of IL-6, demonstrating that increased p-fibrinogen in acute phase conditions is regulated at the cellular level. IL-6 significantly up-regulated expression of Golgi-casein kinase Fam20A (6.0-fold), in contrast to CK2, Fam20B and Fam20C which were unchanged, suggesting that Fam20A plays an important role in the hepatocellular response to acute phase conditions, and may phosphorylate fibrinogen *in vivo*. SEM and turbidimetric assays revealed that clots containing a higher proportion of p-fibrin were composed of thinner fibres, with more extensive branching and slower lysis times.

Conclusion: This study demonstrates that increased intracellular kinase activity leads to elevated p-fibrinogen in acute phase conditions. The observed alterations to clot phenotype suggest phosphorylation of fibrinogen may help to stem bleeding following trauma, but may also have serious implications in the development of thrombosis.

Disclosure of Interest: None declared.

AS004

Mechanisms by which DNA, histones and neutrophil extracellular traps stabilise clots towards mechanical and fibrinolytic breakdownVarjú¹, Longstaff C², Szabó L³, Varga-Szabó VJ¹, Farkas ÁZ¹, Komorowicz E¹ and Kolev K¹¹Department of Medical Biochemistry, Semmelweis University, Budapest, Hungary; ²National Institute for Biological Standards and Control, South Mimms, UK; ³IMEC, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary

Background: Neutrophil extracellular traps (NETs) are meshworks of DNA, histones and granular enzymes forming supplementary scaffolds in thrombi that can be dismantled by heparins. NETs as well as isolated DNA and histones are known to promote thrombus formation and inhibit lysis by tissue-type plasminogen activator (tPA).

Aims: To identify mechanisms of physical and fibrinolytic clot stabilisation by DNA and histones and optimise strategies for modulation (e.g. with heparins, activated protein C (aPC) and DNase).

Methods: DNA $\pm$ histones or phorbol-myristate-acetate (PMA)-activated neutrophils in plasma clots were studied by scanning electron microscopy, fibrinolysis assays and rheometry. Modulation of thrombin (IIa) or FXa inhibition by antithrombin (AT) $\pm$ heparin (UFH, LMWH) was investigated with coagulometry and kinetic assays.

Results: DNA and histones increased median fiber diameters in plasma clots from 108 to 121 and 119 nm, respectively. Histones alone protected IIa from AT inhibition, and IIa and Xa from AT inhibition by neutralising heparin with IC₅₀ of $< 10 \mu\text{g mL}^{-1}$ histones against both UFH and LMWH. In rheometry studies, clots were stabilised by histones and weakened by DNA; histone effects were neutralised by UFH but potentiated by LMWH. DNA and histones slowed tPA-induced fibrinolysis but had no effect on plasmin inactivation by serpins. NETs from PMA-activated neutrophils also inhibited lysis,

which could be reversed by the addition of DNase, while aPC –known to cleave histones– had no effect.

Conclusion: DNA and histones alter the fibrin architecture in plasma clots indirectly (acting through IIa, Xa and AT with and without heparins) and directly by affecting fibrin structure, stability and fibrinolysis. Interactions of histones with UFH and LMWH are not simply related to charge but indicate complex structure relationships and fibrin binding. Cleavage of NET-bound histones is inefficient and has minor effects on fibrinolysis, while DNases enhance effectiveness of tPA.

Disclosure of Interest: None declared.

Platelets and bacteria

AS005

Thrombus formation caused by invading pathogens: the role of platelets

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Given their small size platelets are emerging as being one of the most important entities in the bloodstream. Not only do they play a key role in maintaining thrombosis and haemostasis, platelets also play a critical role in orchestrating the immune response. Being the first cell at the site of injury they are perfectly placed to assess the extent of the damage and recruit immune cells as is necessary. As a first line of defence platelets can act as primitive immune cells themselves by interacting with invading pathogens. Unlike physiological platelet agonists that confer specificity for a platelet receptor, bacteria can engage several platelet receptors at the same time. Binding maybe as a result of a direct interaction when a bacterial adhesin binds directly to a platelet receptor or other surface expressed component on the platelet. Alternatively an indirect interaction occurs when a bacterial adhesin binds to a plasma protein or other soluble elements of the immune system such as immunoglobulins and complement proteins which bridge the bacteria to a specific receptor or other expressed component on the platelet surface. Either way the end result is platelet activation. A major limitation in our current understanding of platelet bacterial interactions stems from the fact that the majority of previous studies have been carried out under static or non-physiological conditions. It has been argued in the literature that data obtained *in vitro* using static binding assays or stirring may not be relevant to the fluid dynamic environment encountered in the vasculature. There are a growing number of papers in the literature in the last number of years suggesting that the local fluid environment of the circulation critically affects the molecular pathways of cell-cell interactions. This paper will discuss findings of platelet bacterial interactions obtained using fluid shear conditions that represent physiological conditions *in vivo* and the lessons learned.

Disclosure of Interest: None declared.

AS006

CLEC-2 is required for the activation of mouse platelets by bacterial DNA mimetics

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Background: Short nuclease-resistant phosphorothioate synthetic CpG motif-bearing oligonucleotides (CpG ODNs) mimicking bacterial DNA display potent immunostimulatory activity and are therefore being used in clinical trials as vaccine adjuvants. Cellular uptake and activation depends on the interaction of CpG ODNs with the C-type lectin receptor DEC-205 and subsequent stimulation of the Toll-like receptor 9 (TLR9) and *myeloid differentiation primary response 88* (MyD88) signaling cascade. Platelets express TLR9, MyD88, and the *C-type lectin-like receptor 2* (CLEC-2). However, the impacts of CpG ODNs on platelet function have been elusive.

Aims: To evaluate whether CpG ODNs affect platelet activation and thrombus formation via CLEC-2 and TLR9.

Methods: We incubated washed platelets or whole blood from TLR9-, MyD88- or CLEC-2- deficient mice with CpG ODNs. We performed platelet aggregometry, flow cytometric binding and platelet activation assays as well as signal transduction analyses. Thrombus formation and fibrin generation were also analyzed by intravital microscopy in mouse microcirculation upon intravenous injection of CpG ODNs.

Results: We show that CpG ODNs bind on platelet surface and are internalized. They activate platelets and induce their aggregation. TLR9- or MyD88-deficient platelets aggregated normally in response to CpG ODN. Interestingly, platelets deficient for the C-type lectin receptor CLEC-2 were unable to capture and internalize CpG ODN. CLEC-2 deficiency abolished CpG ODN-induced platelet activation and aggregation. CpG ODN stimulated CLEC-2 dependent tyrosine kinase pathway and Syk phosphorylation. *In vivo*, intravenously injected CpG ODN interacted with platelets adhered to laser injured arteriolar endothelia and promoted fibrin generation and thrombus growth.

Conclusion: CLEC-2 mediates CpG ODN uptake and subsequent platelet activation, independently of TLR9, which may serve an important role in the interplay between platelets and immunity.

Disclosure of Interest: None declared.

AS007

Platelet Myd88 enhances cytokine production induced by TLR agonists and *Klebsiella pneumoniae*

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Background: MyD88 is the adaptor protein of TLR4 and TLR9, both essential in host defense to *K. pneumoniae*. Platelets also play a crucial role in resistance to *K. pneumoniae* and express TLR4 and TLR9. While it has been shown that platelets modulate cytokine responses by leukocytes, the exact mechanisms are not fully elucidated.

Aims: To assess the role of platelet TLR signaling, specifically MyD88, in modulation of immune response to TLR4, TLR9 and *K. pneumoniae*.

Methods: Platelet specific Myd88 KO (Plt-Myd88^{-/-}) mice were created by crossing Myd88 floxed mice with mice expressing Cre recombinase under control of the PF4 promoter. Cre-negative littermates, full Myd88^{-/-} and TLR9^{-/-} mice were used as controls. Sepsis was induced by i.v. administration of viable *K. pneumoniae*. *In vitro*, heparinized whole blood was incubated with TLR4 agonist (LPS), TLR9 agonist (CpG ODN) or growth arrested *K. pneumoniae*. After 1 h platelet activation and complex formation were determined by flow cytometry. After 6 h, blood was centrifuged and TNF and CXCL1 were measured in plasma by ELISA.

Results: Whole blood of Plt-Myd88^{-/-} mice produced significantly less TNF compared with blood from littermates in response to *K. pneumoniae*, LPS and CpG. Plt-Myd88^{-/-} blood stimulated with *K. pneumoniae* also released less CXCL1. The TNF response was absent in full Myd88^{-/-} mice and with CpG stimulation also in TLR9^{-/-} mice. *In vivo*, Plt-Myd88^{-/-} mice also had lower plasma TNF levels 2 h after i.v. *K. pneumoniae* infection. CpG induced P-selectin and platelet-monocyte complex (PMC) formation in whole blood, which was reduced in blood from TLR9^{-/-} and Myd88^{-/-} mice but not from Plt-Myd88^{-/-} mice. This suggests that the reduced cytokine response to TLR ligands in case of specific platelet Myd88 deficiency is dependent on other factors than P-selectin and PMC formation.

Conclusion: Platelet Myd88 enhances cytokine production induced by *K. pneumoniae*, LPS and CpG.

Disclosure of Interest: None declared.

AS008

Polyphosphate chain length determines antigenicity of complexes formed with platelet factor 4 (PF4) and PF4-binding to bacteria

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Background: Polyphosphates (polyP) are procoagulant, prothrombotic and pro-inflammatory molecules and interfere with innate immune mechanisms. Platelet factor 4 (PF4) is a platelet protein recently discovered to be involved in bacterial host defense mechanisms by binding to lipid A on bacterial surfaces, hereby undergoing a conformational change which allows binding of anti-PF4/heparin antibodies.

Aims: We hypothesized that PF4 (stored in platelet alpha granules), and polyP (stored in platelet dense granules), may interact with each other in bacterial immune defense. We measure the interaction between PF4 and polyP with different mean chain lengths (3-, 45-, and 75-mers) and investigate whether polyP influence the binding capacity of PF4 to Gram-negative bacteria, consecutive binding of anti-PF4/heparin antibodies and phagocytosis of bacteria.

Methods: Circular dichroism spectroscopy was used to investigate changes in the secondary structure of PF4 induced by different polyP. Binding of known anti-PF4/heparin antibodies to PF4/polyP complexes was assessed by enzyme immunoassays. The energetic characteristics of the PF4/polyP binding interactions were determined by isothermal titration calorimetry. The influence of polyP on PF4-binding to bacterial surfaces and anti-PF4/heparin antibody-mediated bacterial phagocytosis was studied by flow cytometry.

Results: Forty-five and 75-mer polyP formed, at specific stoichiometric ratios, complexes with PF4 and induced conformational changes in PF4 (approx. 40% antiparallel β -sheets) in a similar way as shown for PF4 in PF4/heparin complexes. The exposed neopeptides of PF4/polyP complexes bind human anti-PF4/heparin antibodies obtained

from HIT-patients. PolyP (≥ 45 mers) enhance binding of PF4 to *E. coli*, hereby facilitating bacterial opsonization and, in the presence of human anti-PF4/polyanion antibodies, phagocytosis.

Conclusion: PolyP interact with PF4. An additional biological function of polyP is a potential role as part of an ancient immune defense system recognizing bacteria.

Disclosure of Interest: None declared.

AS009

Shear-resistant binding to von Willebrand factor allows *Staphylococcus lugdunensis* to adhere to the cardiac valves and initiate endocarditis

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Background: *Staphylococcus lugdunensis* (*S. lugdunensis*) is an emerging cause of infective endocarditis and vascular infections. The binding of *S. lugdunensis* to the vessel wall and valve endocardium under flow requires a mechanism for shear stress resistant adhesion.

Aims: In this study, we investigated the role of platelets and von Willebrand factor (vWF) in the initial adhesion of *S. lugdunensis* in vascular infections and endocarditis.

Methods: *S. lugdunensis* adhesion to vWF, collagen and endothelial cells was studied in a parallel flow chamber model in the absence and presence of platelets. *In vivo* adhesion of *S. lugdunensis* was evaluated in a mouse microvasculature perfusion model and a newly developed infective endocarditis model.

Results: *S. lugdunensis*, opposed to other coagulase negative staphylococci, is able to bind to vWF under flow thereby enabling its adhesion to activated endothelial cells and the subendothelial matrix. In inflamed vessels of the mesenteric circulation, vWF mediates the recruitment of *S. lugdunensis* to the vessel wall. VWF is also essential for the development of infective vegetations in a new inflammatory endocarditis mouse model.

Conclusion: *S. lugdunensis* binds directly to vWF, which proved to be essential for withstanding shear forces and for successful adhesion of these bacteria to the vessel wall and the cardiac valves. This mechanism may help explain why *S. lugdunensis* causes more aggressive infections, including endocarditis, compared to other coagulase negative staphylococci.

Disclosure of Interest: None declared.

Genetic basis of platelet disorders

AS010

Megakaryocyte and platelet granule biogenesis

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Platelet membrane-bound secretory granules: α -granules, dense (δ -) granules and lysosomes, originate in megakaryocytes and have distinctive cargo sets. δ -granules contain calcium, polyphosphate, ADP, ATP and serotonin, while α -granules are loaded with proteins that include adhesion molecules, cytokines, coagulation/fibrinolytic proteins, immune modulators and complement, growth and angiogenic factors. Studies of inherited δ -granule deficiencies (e.g. Hermansky-Pudlak syndrome, HPS) and mouse models have linked several genes/proteins

involved in the regulation of vesicle trafficking to δ -granule formation, including components of BLOC (biogenesis of lysosome-related organelles complex) complexes, vesicle-trafficking proteins (VPS33A and AP-3 subunits) and the BEACH domain containing protein LYST. Less is known about α -granule development, where two inherited deficiencies: ARC syndrome (arthrogryposis, renal dysfunction, and cholestasis) and gray platelet syndrome (GPS) are characterized by the absence of α -granules and their cargo in platelets. We demonstrated that in ARC syndrome, caused by loss of function mutations in either *VPS33B* encoding the Sec1/Munc18 (SM) protein VPS33B or *C14orf133* (*VIPAS39*) encoding the VPS33B binding protein VPS16B, platelets lack α -granules. We and others have linked GPS to loss of function mutations in *NBEAL2* encoding a BEACH protein. VPS33B/VPS16B null platelets lack the α -granule membrane protein P-selectin, while NBEAL2 null platelets contain it, suggesting that VPS33B/VPS16B are involved in early α -granule formation while NBEAL2 promotes granule maturation and/or cargo loading. Our observation that *Nbeal2* knockout mice show aberrant megakaryocyte/platelet development but unaltered expression of VPS33B/VPS16B support this model. GPS and ARC platelets lack α -granules and contain δ -granules, while HPS platelets lack δ -granules and contain α -granules, indicating distinct cellular pathways of platelet granule biogenesis.

Disclosure of Interest: None declared.

AS011

Consecutive SLFN14 mutations in 3 unrelated families with an inherited bleeding disorder, thrombocytopenia and secretion defects

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Background: Inherited thrombocytopenias are a rare heterogeneous group of disorders characterised by a low platelet count and sometimes associated with excessive bleeding, ranging from mild to severe. Just over 20 forms of inherited thrombocytopenia have been described to date, however in approximately 50% of patients with an inherited thrombocytopenia a causative gene remains to be identified.

Aims: To study a cohort of patients with inherited thrombocytopenia and excessive bleeding using platelet phenotyping and next generation sequencing to identify novel disease genes.

Methods: Thirty-six patients and 17 family members all displaying a reduced platelet count were recruited to the UK-GAPP (Genotyping and Phenotyping of Platelets) study. All patients had a history of excessive bleeding and all major known causes of inherited thrombocytopenia had been excluded prior to enrolment. Platelet phenotyping and whole exome sequencing was performed on all patients.

Results: Whole exome sequencing identified mutations in the novel gene *SLFN14* in 12 patients from three unrelated families. All patients displayed an analogous phenotype of moderate thrombocytopenia, enlarged platelets, decreased ATP secretion upon stimulation with platelet agonists and a dominant inheritance pattern. *SLFN14* codes for a protein of unknown function, Schlafen family member 14 (SLFN14). Three heterozygous missense mutations predicting

p.K218E, p.K219N, p.V220D substitutions within an ATPase-AAA-4, GTP/ATP binding region were identified in affected but not unaffected family members. Platelets from all three families had a marked reduction in expression of endogenous SLFN14. This corresponded with expression studies in HEK293T cells which demonstrated a significant reduction in all three mutants relative to the wild type protein, suggesting instability.

Conclusion: We have identified mutations in *SLFN14* as causative for an inherited thrombocytopenia and significant bleeding, outlining a fundamental role for SLFN14 in platelet formation and megakaryopoiesis.

Disclosure of Interest: None declared.

AS012

Unravelling rare bleeding and platelet disorders (BPD) using comprehensive phenotyping and genome sequencing

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Background: Rare inherited BPD are heterogeneous and the majority have an undefined molecular basis. Current laboratory investigations are laborious and lack sensitivity and specificity. The BRIDGE-BPD consortium is set up to identify novel genes causing these disorders through a combination of detailed clinical and laboratory phenotyping, genome sequencing and application of novel analysis methods

Aims: Identify novel genes and pathways causing inherited BPDs.

Methods: Nearly 1000 cases with unexplained BPDs have been enrolled from 16 international centres and > 700 have undergone genome sequencing. The Human Phenotype Ontology (HPO) terms library was updated to comprehensively annotate BPD cases and used to record clinical and laboratory phenotypes. A clustering algorithm was developed to identify phenotypically similar cases and identify candidate genes.

Results: A median of 7 HPO annotations per case were recorded and analysis of terms revealed the heterogeneous and complex nature of BPD with 60% of cases having abnormalities recorded in organ systems other than the blood, notably in nervous, immune and skeletal systems. Known and new syndromic disorders (e.g. MYH9 and a new GPS-like syndrome) clustered by HPO phenotype, as did cases with ACTN1 variants, validating the algorithm. Phenotypic clustering analysis led to the discovery of novel BPD genes at 5q31, 5p13, 18q11 and 20q11 including novel causes of macrothrombocytopenia and Hermansky-Pudlak syndrome. Additionally, we replicated recent BPD gene discoveries in ACTN1, GFI1B and RASGRP.

Conclusion: We demonstrate that detailed clinical phenotyping using HPO can cluster similar cases within a heterogeneous BPD cohort. Combined with a novel statistical algorithm and large-scale genotyping this approach has led to the discovery of novel BPD genes and could also be applied to other rare disorders with genetic and phenotypic heterogeneity. Our discoveries contribute to understanding of genes controlling platelet formation and haemostasis.

Disclosure of Interest: None declared.

AS013

A dominant gain-of-function mutation in a tyrosine kinase gene causes a new gray platelet-like syndrome with bone pathologies

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Background: The BRIDGE-BPD consortium is a multi-centre collaboration working to identify novel genetic causes of inherited bleeding and platelet disorders (BPD) in about 1000 BPD cases.

Aims: To identify novel genes causing BPD.

Methods: Genome sequencing in combination with an analysis approach based on using the Human Phenotype Ontology (HPO) terms collected for the BPD cases with Mouse Phenotype Ontology terms and megakaryocyte-specific gene expression data identified by the Blueprint consortium (Chen, Science, 2014).

Results: Using this analysis approach, a dominant mutation was found in a kinase gene located at chromosome 20q in a three-generation pedigree with dominant macrothrombocytopenia, bleeding, myelofibrosis, facial dysmorphism and bone pathologies as osteoporosis, premature edentulism and fractures. Increased activation of this kinase was found in cancer but germline mutations related to human disease did not exist. Knockout mice for the kinase only have impaired bone formation with normal platelet counts and no bleeding. Megakaryocytes grown from blood-derived hematopoietic stem cells of 3 affected cases show a defect in proplatelet formation and alpha granule biogenesis, which results in the production of dysmorphic platelets that strongly mimic the Gray Platelet Syndrome (GPS). Electron microscopy of their platelets showed a marked reduction in the number of alpha granules and the presence of large platelets with many vacuoles. Platelets have reduced alpha granule contents and are dysfunctional. A 3D model of the amino acid substitution predicts opening of the kinase self-inhibitory pocket, which is supported by phosphorylation of tyrosine residue present on active open kinase in platelets and megakaryocytes of affected patients and in transfected COS-7 cells.

Conclusion: Our data show that a germline gain-of-function mutation in a kinase causes of a novel syndromic form of dominant GPS with development of myelofibrosis and bone pathologies.

Disclosure of Interest: None declared.

AS014

Mutation in Ets Variant Gene 6 associates with autosomal dominant thrombocytopenia and raised levels of circulating CD34⁺ cells

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Background: Unraveling the defective molecular mechanisms involved in inherited thrombocytopenias is important not only to assure correct treatment but also to allow proper prognosis.

Aims: The aim of this study was to clarify the genetic cause of familial thrombocytopenia with normal platelet size with a suspected dominant mode of transmission but with no known responsible mutation documented.

Methods: We performed a whole-exome sequencing in six family members. *In vitro* experiments were conducted to determine the effects of the identified mutation. Peripheral CD34⁺ cells were differentiated in megakaryocytes. Transcriptional regulatory properties and recruitment of corepressors of the wild-type and mutant were analyzed using Luciferase reporter plasmid.

Results: Exome sequencing identified a heterozygous single nucleotide mutation in Ets-Variant Gene 6 (*ETV6*, c.641C>T; p.P214L). This mutation is located in the central region involved in the recruitment of a repression complex. Screening for *ETV6* mutations in 8 unrelated patients suffering autosomal dominant thrombocytopenia without known genetic etiologies revealed another mutation in *ETV6* leading to a stop codon (c.601_602insC; p.L201Pfs*15).

Both mutations were associated with low repressive *ETV6* activity (residual repressive activity 28% and 0% respectively). p.P214L mutation did not lead to a decreased binding of corepressors (N-CoR, SMRT, Sin3A). p.P214L carrier bone marrow displays dysmegakaryopoiesis with an increased number of megakaryoblasts and hypoblasted megakaryocytes. p.P214L associates with a large expansion of CFU-GM/G/M/MK, a high number of early megakaryocytes and decreased proplatelet formation. Carriers of p.P214L displayed a high level of circulating CD34⁺ cells.

Conclusion: Defects in *ETV6* activity affect the terminal maturation of megakaryocytes and is responsible of autosomal dominant thrombocytopenia.

Disclosure of Interest: None declared.

New alternatives for treating bleeding disorders

AS015

The potential of bispecific antibodies for treatment of hemophilia A

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There are several barriers in the current treatment for severe hemophilia A. One is need of multiple intravenous infusions. The other is difficulty in the hemostatic treatment for the patients who developed FVIII inhibitor. The third one is high drug cost. In order to overcome these unmet needs, we produced FVIIIa mimicking humanized recombinant bispecific antibody, ACE910, against FIXa and FX. Primate model of acquired hemophilia A demonstrated that the ACE910 was effective on both on-going and spontaneous bleedings. Phase I study were conducted in 64 Japanese and Caucasian healthy adults by single subcutaneous infusion of ACE910. There was no safety issue in the clinical and laboratorial findings including thrombotic parameters such as FDP and D-dimer. The half-life of the ACE910 was approximately 30 days. Furthermore, ACE910 shortened aPTT and increased peak height of thrombin generation dose-dependently in FVIII-depleted plasma. This study also included patient part. In this study, Japanese patients with severe hemophilia A (FVIII:C < 1%, ages 12–59 years) were treated with once-weekly SC ACE910 at one of the following dose levels for 12 successive weeks: 0.3 (C1), 1 (C2) and 3 mg kg⁻¹ (C3) followed by extension study up to 9.5 M. Each cohort included 6 patients (18 patients in total). All adverse events were mild or moderate. No thromboembolic AEs were reported, even when FVIII or bypassing agents were administered as on-demand therapy in the presence of ACE910. Four patients in the C1 and C2, and three patients in the C3 cohort had FVIII inhibitors. The inhibitor titers were 3–111 BU mL⁻¹. The median ABR in the C1, C2 and C3 was

reduced from 32.5 to 2.0, 18.3 to 1.2, and 15.2 to 0, respectively. In conclusion, prophylactic treatment by ACE910 by weekly subcutaneous administration was well tolerated and remarkably decreased the number of bleeding episodes in patients with and without FVIII inhibitor.

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AS016

A recombinant fusion protein linking coagulation factor VIIa with albumin (rVIIa-FP) binds to neonatal Fc receptor and tissue factor *in vitro*

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Background: A recombinant fusion protein linking coagulation factor VIIa with human albumin (rVIIa-FP) was developed to extend the half-life of rVIIa-FP aiming to increase convenience in the treatment of hemophilia patients with FVII deficiency or with FVIII/FIX inhibitors. A comparative study in rats using [³H]-labelled proteins showed a comparable biodistribution pattern of rVIIa-FP compared to rFVIIa, but different kinetics of distribution and elimination consistent with the extended half-life of rVIIa-FP. Binding to neonatal Fc Receptor (FcRn) is an established intracellular recycling mechanism for half-life extension *in vivo*, relevant for albumin and immunoglobulins. Binding to the endothelial protein C receptor (EPCR) expressed on vascular cells and interactions with tissue factor (TF) have also been suggested to influence rFVIIa clearance and tissue kinetics. However, to date these interactions have not been fully characterized.

Aims: In this study we investigated possible binding partners for rVIIa-FP on target cells, to illustrate the biodistribution pattern of rVIIa-FP.

Methods: Binding of rVIIa-FP to FcRn was investigated in a cell-based assay using an FcRn-overexpressing 293F cell line. TF binding of rVIIa-FP was analyzed in the human epidermoid carcinoma cell line A-431. Moreover, interactions of rVIIa-FP with FcRn, TF and EPCR were further characterized using Octet, Surface Plasmon Resonance (SPR) and Isothermal Titration Calorimetry (ITC).

Results: We found that rVIIa-FP binds to A-431 cells and co-localizes with TF. Analysis of receptor binding revealed that rVIIa-FP interacts dose-dependently with EPCR, TF and FcRn.

Conclusion: We conclude that rVIIa-FP, might take an additional route of processing, which can help to explain the observed difference in elimination kinetics between rVIIa-FP and rFVIIa. Thus, the presented findings further support clinical investigation of rVIIa-FP for improved therapeutic effectiveness.

Disclosure of Interest: S. Cattepoel Employee of: CSL Behring AG, A. Gaida Employee of: CSL Behring AG, M. Illi Employee of: CSL Behring AG, R. Spirig Employee of: CSL Behring AG, E. Herzog Employee of: CSL Behring GmbH, J. Chia Employee of: CSL Ltd., A. Verhagen Employee of: CSL Ltd., I. Muir Employee of: CSL Ltd., H. Braley Employee of: CSL Ltd., S. Zollner Employee of: CSL Behring AG.

AS017

Long-term safety and prophylactic efficacy of once-weekly subcutaneous administration of ACE910, in Japanese hemophilia A patients with and without FVIII inhibitors: interim results of the extension study of a phase 1 study

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Background: ACE910 is a humanized bispecific antibody that binds factors IXa and X, thereby mimicking the cofactor function of factor VIII (FVIII). We have previously reported that ACE910 has preferable safety and promising efficacy profiles in a phase 1 study.

Aims: To investigate the safety and efficacy of long-term treatment with ACE910 in hemophilia A patients (pts).

Methods: In the phase 1 study, 18 Japanese hemophilia A pts received once-weekly subcutaneous ACE910 at one of the following dose levels for 12 weeks: 0.3, 1 and 3 mg kg⁻¹. These pts were offered to continue on the extension study, including the option to escalate the dose. The studies were approved by all local site ethics committees. Informed consent was obtained from each patient.

Results: Interim data of the phase 1 and extension study combined are presented in this abstract, with a median follow-up of 9.5 months to date. Age ranged from 12 to 58 years. The number of pts with FVIII inhibitors included was 4, 4 and 3 respectively for each dose level. A total of 16 pts participated in the extension study. The ACE910 dose was escalated in 2 pts from 0.3 to 1 mg kg⁻¹ due to frequent bleeding. Eighty adverse events (AEs) reported in 17 pts were mild or moderate. No thromboembolic AE was reported, even when FVIII or bypassing agents were given concomitantly as on-demand therapy. Anti-ACE910 antibodies screened by electrochemiluminescence immunoassay were developed in 2 pts, which did not affect ACE910 pharmacokinetics or pharmacodynamics.

The annualized bleeding rate (ABR) decreased compared to the ABR prior to ACE910 initiation for both pts with and without inhibitors. The median ABR was reduced from 32.5 to 2.0, 18.3 to 1.2, and 15.2 to 0.0 for the 0.3, 1 and 3 mg kg⁻¹ dose levels, respectively.

Conclusion: The long-term safety and efficacy data show that ACE910 could be a groundbreaking treatment option for hemophilia A pts irrespective of the presence of FVIII inhibitors. In addition to promising efficacy, ACE910 improved the quality of life.

Disclosure of Interest: M. Shima Grant/Research Support from: Chugai Pharmaceutical Co., Ltd., Consultant for: Chugai Pharmaceutical Co., Ltd., H. Hanabusa Grant/Research Support from: Chugai Pharmaceutical Co., Ltd., M. Taki Grant/Research Support from: Chugai Pharmaceutical Co., Ltd., T. Matsushita Grant/Research Support from: Chugai Pharmaceutical Co., Ltd., T. Sato Grant/Research Support from: Chugai Pharmaceutical Co., Ltd., K. Fukutake Grant/Research Support from: Chugai Pharmaceutical Co., Ltd., N. Fukazawa Shareholder of: Chugai Pharmaceutical Co., Ltd., Employee of: Chugai Pharmaceutical Co., Ltd., K. Yoneyama Employee of: Chugai Pharmaceutical Co., Ltd., H. Yoshida Employee of: Chugai Pharmaceutical Co., Ltd., H. Takahashi Consultant for: Chugai Pharmaceuti-

cal Co., Ltd., K. Nogami Grant/Research Support from: Chugai Pharmaceutical Co., Ltd., Consultant for: Chugai Pharmaceutical Co., Ltd.

AS018

Pharmacodynamics and pharmacokinetics of TFPI-neutralizing antibody (BAY1093884) in cynomolgus monkeys and prediction of human dose

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Background: BAY1093884 is a fully human monoclonal antibody against tissue factor pathway inhibitor (TFPI) developed as a bypass agent for hemophilia patients with inhibitors. *In vitro*, BAY1093884 potentially binds to human, mouse and monkey TFPI with a KD of ≤ 50 pM.

Aims: The objective of this study is to find a pharmacodynamic (PD) biomarker after intravenous (i.v) and subcutaneous (s.c) administration of BAY1093884 into normal monkeys, and to develop dosing strategy for BAY1093884 that may be used for the treatment of hemophilia.

Methods: We have employed FXa captured TFPI ELISA assay to quantify functional free TFPI levels and a diluted prothrombin time (dPT) to measure TFPI anticoagulant activity.

Results: The IC₅₀ (concentration that inhibits 50% of TFPI levels and dPT clotting time) of BAY1093884 was determined to be 4.65 and 6.19 nM in monkey plasma. The pharmacokinetic (PK) profile of BAY1093884 and the PD effects on dPT clotting time and free-TFPI levels were assessed after i.v and s.c administration of BAY 1093884 to female cynomolgus monkeys at doses of 5 and 20 mg kg⁻¹. Free TFPI concentrations in plasma decreased rapidly and returned to baseline in a dose-dependent manner. The dPT clotting time was also shortened and correlates to free TFPI levels and drug concentration in plasma over the time after administration of BAY1093884 to monkeys. These results clearly demonstrate the relationship between PD activities (dPT clotting time and free TFPI levels) and the drug concentration. The estimated efficacious concentration of BAY1093884 was determined to be 6 µg mL⁻¹ or 40 nM. Moreover, BAY1093884 exhibited non-linear PK, and a target-mediated drug disposition (TMDD) model was used to characterize BAY1093884 vs. TFPI concentration response relationship.

Conclusion: From this modeling application, we conclude that mechanism-based PK/PD binding model is useful for predicting human response to BAY1093884. For the first in man study, the measurement of free-TFPI will be included as part of dose escalation design.

Disclosure of Interest: J.-M. Gu Employee of: Bayer HealthCare Pharmaceuticals, E. Ho Employee of: Bayer HealthCare Pharmaceuticals, X.-Y. Zhao Employee of: Bayer HealthCare Pharmaceuticals, T. Schwarz Employee of: Bayer HealthCare Pharmaceuticals, J. Schuhmacher Employee of: Bayer HealthCare Pharmaceuticals, B. Subramanyan Employee of: Bayer HealthCare Pharmaceuticals, K. Tran Employee of: Bayer HealthCare Pharmaceuticals, C. Patel Employee of: Bayer HealthCare Pharmaceuticals, M. Koellnberger Employee of: Bayer HealthCare Pharmaceuticals.

AS019

Thrombin generation is increased in plasma from healthy males who have received concizumab, an antibody against tissue factor pathway inhibitor (ExplorerTM2)

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Background: Inhibition of tissue factor pathway inhibitor (TFPI) may be an effective treatment for haemophilia by allowing sufficient thrombin generation by tissue factor (TF), factor (F) VIIa and FXa in the initiation phase of coagulation. Concizumab, an anti-TFPI antibody, was tested in a multi-centre, open-labelled, multiple dosing phase 1 clinical trial (ExplorerTM2) where it was administered subcutaneously to healthy males.

Aims: To investigate if concizumab improves thrombin generation in plasma from healthy males.

Methods: Four healthy males received 250 µg kg⁻¹ concizumab every other day 8 times. Blood was collected at each visit in tubes with 3.2% trisodium citrate and 50 µg mL⁻¹ corn trypsin inhibitor. Thrombin generation was measured in platelet-poor plasma with 1 pM TF and 4 µM phospholipids. Concizumab and free TFPI levels were measured via ELISA. A subset of samples was treated with a neutralising FVIII antibody to mimic haemophilia A inhibitor plasma before measuring thrombin generation.

Results: Thrombin generation parameters of peak thrombin, endogenous thrombin potential and velocity index increased in plasma samples after concizumab dosing compared to baseline (no concizumab) samples. All parameters returned to baseline by trial end. Over the course of the trial, concizumab levels increased, and free TFPI levels decreased. When baseline samples and samples after concizumab dosing were treated with a neutralising FVIII antibody, the presence of concizumab resulted in increased thrombin generation.

Conclusion: Concizumab administration improves thrombin generation in plasma from healthy males. Thrombin generation correlates with concizumab (directly) and TFPI (inversely) levels. In plasma treated with a neutralising FVIII antibody, improved thrombin generation was observed in the presence of concizumab as compared to plasma without concizumab. These findings suggest that concizumab administered to patients with haemophilia should improve thrombin generation.

Disclosure of Interest: E. Waters Employee of: Novo Nordisk A/S, J. Sigh Employee of: Novo Nordisk A/S, M. Ezban Employee of: Novo Nordisk A/S, I. Hilden Employee of: Novo Nordisk A/S.

Women's issue in venous thrombosis

AS020

Antithrombotic therapy to prevent placenta mediated complications

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Several adverse obstetric outcomes are associated with placental insufficiency. Examples include fetal growth restriction (FGR), preeclampsia and fetal death. Taken together, these disorders affect between 5% and 10% of pregnancies. Placental insufficiency can be due to abnormal placental development, placental damage or both. In some cases, this is thought to be due to abnormal blood flow, with or without thrombosis in the uteroplacental circulation. In turn, this leads to infarction of placental tissue and subsequent obstetric complications. This hypothesis was initially fueled by observations in women with an-

tipospholipid syndrome (APS), which led to the use of anticoagulant therapy in women with the condition. However, anticoagulant therapy is now widely used in women without APS in hopes of improving obstetric outcomes. Despite widespread use, proof of efficacy is lacking. Studies assessing the use of anticoagulant therapies including aspirin and heparins in hopes of preventing placenta mediated complications will be reviewed. Important subsets of women including APS, heritable thrombophilias and those with prior obstetric disorders will be discussed. Other potentially beneficial effects of anticoagulant therapy including decreased inflammation and enhanced placental growth and development will be reviewed. Finally, recommendations for evidence-based practice and future research will be emphasized.

Disclosure of Interest: None declared.

AS021

Time to live birth in women with recurrent miscarriage and inherited thrombophilia

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Background: We recently reported on the time to conception and time to live birth in the first subsequent pregnancy in women with unexplained recurrent miscarriage. Time to conception was about 2-fold shorter in women who carried factor V Leiden as compared to non-carriers. The prognosis of women with inherited thrombophilia and recurrent miscarriage after multiple pregnancy attempts however, is unknown.

Aims: To evaluate the association between inherited thrombophilia and time to live birth after multiple pregnancy attempts over an extended follow-up period in women with unexplained recurrent miscarriage.

Methods: Cohort study of women with two or more miscarriages ($n = 364$), who participated in the ALIFE study (Kaandorp, NEJM 2010). Data of subsequent pregnancies were collected using questionnaires between November 2012 and September 2014. Primary outcome measure was time to first live birth since the last miscarriage prior to randomization in the ALIFE study. The probability of a live birth was estimated using competing risks analyses, where reaching the age of 46 years was considered a competing risk for a live birth.

Results: Follow-up data were available for 271 of 364 (74%) women, with a median follow-up of 84 months (range 15–169 months). Thrombophilia was considered absent if not available ($n = 42$). Inherited thrombophilia was diagnosed in 41/271 (15%) women. The cumulative probability of live birth for the full cohort was 15%, 55%, 77% and 81% after 1, 2, 5 and 10 years, respectively. Inherited thrombophilia was not associated with time to live birth in univariate and multivariate analyses (adjusted hazard ratio 1.15, 95% confidence interval 0.77–1.72).

Conclusion: The prognosis of women with unexplained recurrent miscarriage to achieve a live birth after multiple pregnancy attempts is not affected by inherited thrombophilia. This may reflect an increased number of miscarriages after an initial shorter time to conception in these women.

Disclosure of Interest: P. de Jong, S. Kaandorp, R. Kool, B. Hutten, and M. Goddijn: None declared; S. Middeldorp Grant/Research Support from: Research grants from GSK and Aspen, Speaker Bureau of: Speaker fees from GSK.

AS022

Hormonal therapy and the risk of recurrent venous thromboembolism in women receiving anticoagulant treatment

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Background: Women of childbearing potential who receive anticoagulation with coumarin derivatives require adequate contraception because these agents may cause both fetal bleeding and severe embryopathy. In women using anticoagulants, physicians are reluctant to prescribe estrogen-containing therapies because of the documented increased risk of venous thromboembolism (VTE), despite an absence of data in the literature on the risk of recurrent VTE during anticoagulation.

Aims: To compare the incidence of recurrent VTE with and without concomitant hormonal therapy in women aged ≤ 60 years randomized to rivaroxaban or enoxaparin/vitamin K antagonist (VKA) in the randomized EINSTEIN DVT and PE trials.

Methods: Incidence-densities in % per year were computed for the 'on' and 'off' hormonal therapy periods. Cox regression models were fitted, with hormonal therapy (on vs. off) as a time-dependent variable to derive the hazard ratio (HR) for its effects on recurrent VTE.

Results: A total of 1888 women aged ≤ 60 years were included in this analysis, of whom 475 received hormonal therapy (306 estrogen-containing, 217 progestin-only, including 48 who used both therapies one after the other). The accumulated patient-years at risk on hormonal therapy were 187.5 years (estrogen-containing: 109.5 years; progestin-only: 78.0 years), and 811.0 years for without use. Seven recurrent VTE events occurred during hormonal therapy and 38 events occurred in the period without use. Crude incidence-densities were 3.7% per year and 4.7% per year with and without hormonal therapy. The crude incidence-densities for estrogen-containing and progestin-only therapy were 3.7% per year and 3.8% per year. The HR, stratified for age and adjusted for pre-randomization use of hormonal therapy, active cancer and assigned treatment was 0.56 (95% confidence interval 0.23–1.39).

Conclusion: Use of either estrogen-containing or progestin-only therapy was not associated with an increased risk of recurrent VTE in women receiving either rivaroxaban or enoxaparin/VKA.

Disclosure of Interest: I. Martinelli: None declared, A. Lensing Employee of: Bayer HealthCare, J. Beyer-Westendorf Grant/Research Support from: Bayer HealthCare, Daiichi Sankyo, Pfizer, Boehringer Ingelheim, Novartis and LEO Pharma, M. Trajanovic Employee of: Bayer HealthCare, M. Gebel Employee of: Bayer HealthCare, P. Lam Employee of: Bayer HealthCare, M. Prins Grant/Research Support from: Bayer, Sanofi-Aventis, Boehringer Ingelheim, GSK, Daiichi Sankyo, LEO Pharma, ThromboGenics and Pfizer.

AS023

The proportion of nondiagnostic computed tomographic pulmonary angiography and ventilation/perfusion lung scans in pregnant women with suspected pulmonary embolism: a systematic review

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Background: Both computed tomographic pulmonary angiography (CTPA) and ventilation/perfusion (VQ) lung scanning are used to investigate patients with suspected pulmonary embolism (PE), however, controversy exists regarding which is better to use in pregnant women. The chance of obtaining a nondiagnostic result is important when choosing an imaging test in patients with suspected PE. While CTPA has a lower proportion of nondiagnostic scans compared to VQ in the non-pregnant population, the proportion of nondiagnostic scans in pregnant women is unknown for either modality.

Aims: To determine the proportion of nondiagnostic CTPA and the proportion of nondiagnostic VQ scans in pregnant women with suspected PE.

Methods: We searched the MEDLINE, EMBASE, and Cochrane databases for relevant studies. We included retrospective or prospective studies (including abstracts) that enrolled pregnant or postpartum women who underwent CTPA or VQ lung scanning for suspected PE and reported the number of nondiagnostic scans for either test. Pooled proportions of nondiagnostic scans were calculated using a random effects model for CTPA and for VQ. Predefined subgroup analysis included antepartum vs. postpartum, dose of CT contrast agent and dose of perfusion tracer.

Results: Twenty-two studies including 2391 scans were eligible for inclusion. Sixteen studies described 1226 CTPA, and 13 studies described 1165 VQ lung scans. The pooled proportion of nondiagnostic scans using CTPA was 12.5% (95% CI 7.5–18.4, $I^2 = 86\%$), and using VQ was 11.5% (95% CI 5.3–19.6, $I^2 = 93\%$). Heterogeneity was not explained by any of the predefined subgroups.

Conclusion: The proportion of nondiagnostic scans is low in women undergoing either CTPA or VQ, and should not be a factor in determining which imaging modality is chosen to investigate PE in the pregnant population.

Disclosure of Interest: A. Parker, G. Alotaibi, and S. Takach Lapner: None declared; C. Wu Consultant for: Leo Pharma and Pfizer.

Modulation of atherosclerosis

AS024

The hemostatic system as a modulator of atherosclerosis

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Atherosclerosis is a chronic inflammatory disease of the arteries, responsible for athero-thrombotic complications leading to vascular death, myocardial infarction, ischemic stroke as well as ischemia in organs like limbs and kidneys. The co-localization of inflammation and coagulation in atherosclerosis was already recognized in the 19th century; the question was, whether thrombosis was cause or consequence of vascular inflammation and remodeling? In either case, clotting is a wound healing system, containing the inflammatory process and supporting vascular regeneration. Many studies demonstrate extensive involvement of coagulation products in atherosclerosis: coagulation proteases (factors VIIa, Xa, thrombin) that signal through protease activated receptors, anticoagulants like tissue factor pathway inhibitor that dampen such signals, fibrin and its cleavage products that directly affect cellular activity in the vessel wall. Locally expressed

coagulation proteins including factor VII interact with tissue factor to drive local thrombin and fibrin formation. Together, these elements may be protective up to the point that natural defense mechanisms fail to limit the extent of clotting and inflammation. Diminished natural anticoagulant properties on the endothelium (thrombomodulin and endothelial cell protein C receptor) accelerate the occurrence of athero-thrombotic events at advanced age. In blood, coagulation proteases and platelets affect, in concert with leukocytes and microvesicles, the prothrombotic effect; all elements that enhance thrombin and fibrin formation in blood (thrombophilic factors) also increase the risk of atherothrombosis. Anticoagulants attenuate the prothrombotic and potentially atherogenic effects of coagulation. Non vitamin K antagonist oral anticoagulants (NOAC) like dabigatran markedly limited atherosclerosis in different mouse studies. The clinical consequences of longterm anticoagulant treatment merit further study.

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AS025

Adoptive transfer of antigen-induced specific regulatory T cells (TREG) protects against atherosclerotic lesion formation in B6;129S-Ldlr (tm1her)Apob(tm2sgy)/J mice

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Background: Atherosclerosis is an inflammatory disease in which plaque builds up inside the arteries. Natural regulatory T cells have a protective role in the progression of atherosclerosis, however, the mechanism of adoptively transferred antigen-induced Treg cells on atherosclerotic lesion formation remains unclear.

Aims: To investigate the mechanisms of adoptively transferred Treg isolated from the blood of antigen-immunized mice on atherosclerotic lesion formation in B6;129S-Ldlr^{tm1her}Apob^{tm2sgy}/J mice.

Methods: Antigenic epitopes derived from Apolipoprotein B (ApoB) (AA688–707) designated as epitope A, human heat shock protein (HSP)60 (AA303–312) as H^h, mycobacterium HSP60 (AA253–268) as H^m and complement component 5a receptor (C5aR) (AA1–31) as R were incorporated into a dendroaspilin scaffold to generate AH^hH^mR as a multiple epitopic construct. The Treg cells purified from the blood of immunized mice with this construct were adoptively transferred into B6;129S-Ldlr^{tm1her}Apob^{tm2sgy}/J mice subsequently fed with a high-fat diet for 10 weeks.

Results: Histological analyses demonstrated that after adoptive transfer of antigen-induced specific Treg cells, the mice showed significant reduction in the size of atherosclerotic lesions (48% reduction, $P < 0.001$) when compared with that in control (Treg cells from immunized mice with dendroaspilin scaffold). This effect on lesion reduction was associated with a shift in the cellular composition of plaques towards decreased inflammatory cell and increased production of anti-inflammatory cytokines and decreased secretion of proinflammatory cytokines demonstrated in plasma and in supernatant of stimulated spleen cells.

Conclusion: Antigen-induced specific Treg cells significantly reduced atherosclerotic lesion by suppressing T effector cell proliferation and the macrophage-derived foam cell formation towards inhibition of atherosclerotic lesion formation. This approach may offer attractive opportunities on the cell-based therapy for the treatment of atherosclerosis.

Disclosure of Interest: None declared.

AS026

Disruption of semaphorin 7A decreases plaque size induced by disturbed flow and confers protection against the development of atherosclerosis

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Background: Atherosclerosis is an inflammatory disease preferentially developed in the areas of arteries with arches and branches, where the flow is disturbed (D-flow). Increasing evidence suggests that Semaphorin7A (Sema7A) plays important roles in several pathophysiological processes.

Aims: We aimed to investigate the role of Sema7A in the development of atherosclerosis, and particularly, its effect on D-flow-induced pro-atherogenic process.

Methods: ApoE^{-/-} mice were crossed with Sema7A^{-/-} mice to generate Sema7A^{-/-}ApoE^{-/-} mice and Sema7A^{+/+}ApoE^{-/-} mice. High fat diet was used to induced atherosclerotic plaque. Meanwhile, we used partial carotid ligation model to investigate whether the varied inhibition of plaque formation at different locations of aorta by deletion of Sema7A is related to D-flow.

Results: Lipid deposition in the whole aorta was decreased by 51.18% ($P = 0.0024$) in Sema7A^{-/-}ApoE^{-/-} mice ($5.01 \pm 1.04\%$, $n = 13$) compared to Sema7A^{+/+}ApoE^{-/-} control mice ($10.26 \pm 1.15\%$, $n = 13$), with more striking difference in aorta arch (59.16%, $P = 0.0005$). After PCL surgery, we showed that Sema7A expression in the LCA was 2.6-fold higher ($P = 0.0237$, $n = 3$) in D-flow condition compared to right carotid artery (RCA). We also showed that plaque size in the LCA under D-flow was decreased by 50.74% ($P = 0.0072$) in ApoE^{-/-}Sema7A^{-/-} mice ($0.2411 \pm 0.05265\%$, $n = 6$) compared to Sema7A^{+/+}ApoE^{-/-} control mice ($0.4894 \pm 0.05186\%$, $n = 6$). Mechanism studies showed that deletion of Sema7A reduced leucocyte rolling on the vascular wall in response to LPS stimulation, decreased accumulation of macrophages (58.437%, $P = 0.0010$), CD4⁺ cells (51.31%, $P < 0.0001$) and dendritic cells (60.4%, $P < 0.0001$), and enhanced collagen content ($P < 0.0022$) and vulnerability index ($P < 0.0001$) in ApoE^{-/-} mice.

Conclusion: These results demonstrate that genetic deletion of Sema7A attenuates plaque formation likely by impairment of the migration and accumulation of macrophages, T cells and dendritic cells in the vascular wall, which may correlates with D-flow condition.

Disclosure of Interest: None declared.

AS027

Changes in peripheral immune markers in response to immune tolerance and its correlation with progression of atherosclerosis in ApoBtm2Sgy Ldlrtm1Her miceRao TL¹, Philip S¹, Ponnusamy T¹, Lu X², Kakkar VV^{1,2} and Mundkur L¹*¹Molecular Immunology, Thrombosis Research Institute, Bangalore, India; ²Molecular Immunology, Thrombosis Research Institute, London, UK*

Background: Adaptive immune response to atherogenic self antigens initiates an inflammatory autoimmune response during atherogenesis. We have earlier shown that oral administration of a multiantigenic molecule (AHC) expressing peptides derived from ApoB100 (AA688–707), human HSP60 (AA153–162) and outer membrane protein of *Chlamydia pneumoniae* (AA67–74 and 283–291) induces tolerance to individual peptides and controls the development of atherosclerosis.

Aims: To study the changes in lymphocyte and monocyte population in spleen in response to immune therapy and its association with atherosclerosis

Methods: ApoB^{tm2Sgy} Ldlr^{tm1Her}/J KO mice (5–6 weeks) were given five oral doses of AHC molecule to induce immune tolerance followed by high-fat diet feed to induce atherosclerosis. Animals were sacrificed after 12, 18 and 30 weeks to study the disease progression.

Results: Histological analysis revealed 56.6% ($P = 0.001$) reduction in plaque in aortic sinus of AHC treated mice at 12 weeks, 22.8% ($P = 0.001$) at 18 weeks and 23.4% ($P = 0.01$) after 30 weeks compared to untreated control. Longer duration of disease induction reduced the effect of tolerance. We observed a significant increase in regulatory T cells in the spleen at 12 weeks (72.9%, $P = 0.005$) and 18 weeks (66.6%, $P = 0.04$) but the Treg cells were comparable between the groups at 30 weeks. The proportion of Treg cells reduced in the AHC treated animals from 12 to 18 and 30 weeks. We observed a concomitant increase in T cells secreting IFN- γ and IL17 with disease progression. The proportion of Treg cells showed a significant inverse correlation with aortic lesion.

Conclusion: Our results suggest that oral tolerance induces regulatory T cells which can balance the adaptive immune response. Prolonged hypercholesterolemia shifts this balance and the effect of tolerance is reduced. Optimizing immune tolerance could induce long term protection against the disease.

Disclosure of Interest: None declared.

AS028

Bone morphogenetic protein (BMP)-7 upregulates adhesive and migratory properties of human monocytic cells via beta2 integrin-BMP receptor type II (BMPRII) crosstalkSovershaev T^{1,2,3}, Unruh D³, Hansen J-B^{1,2,4}, Bogdanov V³ and Sovershaev M^{2,5}*¹K.G. Jebsen Thrombosis and Expertise Centre (TREC);**²Hematological Research Group, Department of Clinical**Medicine, the Faculty of Health Sciences, University of Tromsø,**Tromsø, Norway; ³Division of Hematology/Oncology,**Department of Internal Medicine, University of Cincinnati**College of Medicine, Cincinnati, OH, USA; ⁴Division of Internal**Medicine; ⁵Section for Medical Biochemistry, Department of**Laboratory Medicine, University Hospital of Northern Norway, Tromsø, Norway*

Background: BMP-7 is enriched in the plaque and increases monocyte pro-coagulant activity by enhancing Tissue Factor (TF) expression. It was recently reported that BMP-2/-4 act as chemotactic agents for monocytes, which requires BMPRII expressed on monocyte surfaces. The mechanisms responsible and/or BMP-7's ability to modulate monocyte behavior are not known.

Aims: To assess BMP-7's ability to upregulate adhesive and migratory properties of human monocytes.

Methods: Chemokinesis, adhesion, and chemotaxis of BMP-7 treated THP-1 cells were analyzed using live-cell imaging, orbital shear, and Boyden chamber assays, respectively. Surface presentation of $\beta 2$ (CD18) integrins, their co-localization with BMPRII, and phosphorylation status of AKT & Focal Adhesion Kinase (FAK) were studied. One-way ANOVA was used to assess significance, set at $P < 0.05$.

Results: Exposure of THP-1 cells to BMP-7 (300 ng mL^{-1}) enhanced their chemokinetic properties: 1.25 fold increase in crawling distance and 1.38 fold increase in cell velocity over vehicle. Under orbital shear, adhesion of BMP-7 treated THP-1 cells to microvascular endothelial cell (MVEC) monolayers was also increased (6.27 fold over vehicle); moreover, BMP-7 enhanced THP-1 chemotactic properties (5.55 fold increase in the number of cells migrated through MVEC monolayers towards MCP-1 at 10 ng mL^{-1}). Critically, BMP-7 rapidly increased co-localization of $\beta 2$ integrins with BMPRII (1.45 fold over vehicle, 5 min post-treatment), enhanced surface presentation of $\beta 2$ integrins

in its active conformation (2.18 fold over vehicle, 30 min post-treatment), and increased the levels of AKT and FAK phosphorylation.

Conclusion: BMP-7 upregulates adhesion and migration of human monocytic cells via a novel mechanism evidently involving $\beta 2$ integrin-BMPRII crosstalk on cell surfaces, leading to $\beta 2$ integrin activation. BMP-7 may thus serve as a critical factor contributing to plaque thrombogenicity, as well as its monocyte-mediated destabilization.

Disclosure of Interest: None declared.

Coagulation, fibrinolysis, immunity and inflammation

AS029

Cross-talk between coagulation and fibrinolysis

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As components of the host defense system, the response of both the coagulation and fibrinolytic cascades are coordinated to ensure full recovery from injury or infection. One link is carboxypeptidase B2 (CPB2, TAFIa) that is activated by the thrombin/thrombomodulin (TM) complex and less efficiently by plasmin from a circulating zymogen, proCPB2. Its substrates include fibrin leading to less plasmin generation and lower fibrinolysis, and inflammatory mediators such as bradykinin and complement anaphylatoxins, C3a and C5a, that are inactivated. The *Cpb2*^{-/-} mouse is fertile and has no phenotype until challenged. Recently we have been focusing on the role of CPB2 in infection and inflammation, using pharmacological interventions in models in the *Cpb2*^{-/-} mouse. In a model of polymicrobial sepsis, *Cpb2*^{-/-} mice were protected compared to wild type (WT). Both C5a and fibrin were CPB2 substrates in this model, but neither was the key substrate leading to the observed protection. Importantly, treatment with a C5a receptor antagonist improved the outcomes for both WT and *Cpb2*^{-/-} mice. In contrast, a C3a receptor antagonist worsened outcomes for WT and *Cpb2*^{-/-} and eliminated the survival advantage seen in the *Cpb2*^{-/-} mice. Thus, C3a and C5a are both relevant targets and have opposite effects in polymicrobial sepsis. Because of the observed complement effects, we have developed a model of Shiga toxin (Stx) and lipopolysaccharide (LPS)-induced hemolytic uremic syndrome (HUS). To develop HUS both Stx and LPS administration are necessary. *Cpb2*^{-/-} mice have worse outcomes than WT in this model. These data confirm that the role of CPB2 is not confined to modulating fibrinolysis. Instead CPB2 is a critical regulator of inflammation via its inactivation of anaphylatoxins, and the thrombin/TM activation of proCPB2, along with activation of protein C, represents a homeostatic response to regulate thrombin's inflammatory activities.

Disclosure of Interest: J. Morser Shareholder of: Gemmus Pharmaceuticals, Consultant for: Gemmus Pharmaceuticals.

AS030

Imaging analyses of coagulation-dependent initiation of fibrinolysis on activated platelets and its modification by TAFI

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Background: Employing intra-vital confocal microscopy, we reported that the process of platelet phosphatidylserine (PS) exposure, fibrin formation and lysine binding site-dependent plasminogen (plg) accumulation took place only in the center of the thrombus but not in its

periphery. These suggest that coagulation and fibrinolysis are closely related and finely regulated.

Aims: To analyze *in-vitro* the spatiotemporal regulatory mechanisms of coagulation and fibrinolysis.

Methods: Fibrin network formation and the following lysis were analyzed in the experiment employing diluted platelet-rich plasma supplemented with fluorescently labeled coagulation- and fibrinolytic-factors using Confocal Laser Scanning Microscopy.

Results: Tissue factor triggered platelet PS-dependent fibrin network formation. Fibrin network structure was uneven and denser at the sites of coagulation initiation regions (CIRs) on PS exposing platelets. When tissue type plasminogen activator (tPA; 7.5 nM) was supplemented, CIRs appeared to be the primary accumulation sites for labeled plg (50 nM) as well as tPA, from where fibrinolysis started and gradually expanded to the peripheries. The lysis time at CIRs and their peripheries were 25.9 ± 10.2 and 35.2 ± 7.4 min (mean $\pm$ SD, $n = 50$ from 5 independent experiments) from TF addition, respectively. Recombinant human soluble thrombomodulin (rhsTM; 1.66 nM) attenuated CIR-dependent plg accumulation, and strongly delayed fibrinolysis at CIRs (approx. 5 times). Carboxypeptidase inhibitor (10 μ M) enhanced CIR-dependent fibrinolysis initiation, and entirely abrogated rhsTM-induced delay of fibrinolysis.

Conclusion: Our study for the first time directly presents the crosstalk between coagulation and fibrinolysis, which takes place on the activated platelets surface and is further controlled by thrombin activatable fibrinolysis inhibitor (TAFI).

Disclosure of Interest: None declared.

AS031

Activation of prochemerin by factor XIa represents a new molecular link between innate immunity and blood coagulation

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Background: Chemerin is a chemoattractant and an adipokine that plays a role in innate immunity as it promotes the migration of various types of innate immune cells. Chemerin circulates in blood as inactive prochemerin (Chem163S). Prochemerin is activated by a series of C-terminal proteolytic steps resulting in diverse chemerin isoforms with different levels of activity. The fully active form, chem157S, is produced by plasmin cleavage at Lys¹⁵⁸ (chem158K) followed by removal of the C-terminal lysine by carboxypeptidase B2 (CPB2 or TAFIa) or carboxypeptidase N (CPN).

Aims: Identify coagulation proteases that process the C terminus of chemerin.

Methods: We screened a panel of proteases to identify proteases other than plasmin that process the C terminus of chemerin by mass spectroscopic (MS) analysis of their products. Activated factor XI (FXIa) can cleave chem163S. We produced human Chem163S and a mutant with a 6his-tag on their N-terminus (6his-Chem163S and 6his-Chem163S_{R162A}). We performed FXIa and plasmin cleavage of purified 6his-Chem163S or 6his-Chem163S_{R162A} in a purified system and in plasma. Products were analyzed by ELISA, Western and MS.

Results: FXIa cleaved 6his-Chem163S generated a novel chemerin form, 6his-Chem162R, as well as 6his-Chem158K, but converted 6his-Chem163S to 6his-Chem158K much slower than plasmin. Both FXIa and plasmin proteolyzed 6his-Chem163S_{R162A} into 6his-Chem158K, showing that cleavage of Chem163S at R¹⁶² and K¹⁵⁸ occurred simultaneously and processing at R¹⁶² was not required for cleavage at K¹⁵⁸. Activation of human plasma by kaolin also led to cleavage of 6his-Chem163S, which was abolished in FXI-deficient plasma.

Conclusion: This study identified FXIa as a protease that can cleave Chem163S, resulting in Chem162R, a novel chemerin form, and Chem158K, which can be further processed to the most active chemerin form. Processing at both sites, R¹⁶² and K¹⁵⁸, occurs simultaneously. Cleavage of chem163S in plasma occurs when the contact phase is activated and is dependent on FXIa.

Disclosure of Interest: None declared.

AS032

Intravenous thrombolysis generates large amounts of bradykinin in stroke patients: neurotoxic impact

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Background: Fibrinolysis using recombinant tissue-type plasminogen activator (tPA) remains the only approved treatment for acute ischemic stroke. *In vitro*, tPA is able to generate bradykinin by a plasmin dependent mechanism. Notably, bradykinin is a pro-inflammatory peptide that plays a deleterious role during ischemic stroke.

Aims: (i) To study bradykinin generation during fibrinolysis in humans and (ii) to investigate the role of tPA-induced bradykinin generation in a mouse model of ischemic stroke.

Methods: Blood samples were obtained from ischemic stroke patients ($n = 8$). Bradykinin concentrations were measured by ELISA and by Western Blot of its (cleaved) precursor, high molecular weight kininogen. Ischemic stroke was induced in mice by *in situ* injection of thrombin.

Results: Intravenous fibrinolysis generated large amount of bradykinin in ischemic stroke patients. *In vitro* studies confirmed that this effect was mediated by plasmin. In mice, pharmacological blockade of the bradykinin B1 (B1R) but not bradykinin B2 (B2R) receptors improved stroke outcome in thrombolized mice. This was associated with a reduced level of pro-inflammatory mediators (ICAM-1, VCAM-1, TNF, IL-1b) in the ischemic brain. Consistently, B1R knockout mice were protected from ischemic stroke. Interestingly, this effect was only observed in mice receiving intravenous tPA, confirming the critical role of exogenous tPA in bradykinin generation during stroke.

Conclusion: tPA-mediated fibrinolysis generates large amounts of bradykinin by a plasmin dependent mechanism. Through B1R activation, this generated bradykinin plays a deleterious role during ischemic stroke by worsening post-stroke inflammation. Therefore, pharmacological blockade of B1R during fibrinolysis appears as a promising adjunctive treatment for acute ischemic stroke.

Disclosure of Interest: None declared.

Antiphospholipid antibodies

AS033

Unravelling the antiphospholipid syndrome: from empiric description to pathogenic mechanisms

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The current approaches for diagnosing the antiphospholipid (aPL) syndrome (APS) are problematic in that they are empirically derived and not mechanistic. The lupus anticoagulant (LA) phenomenon was first described over 60 years ago as an anomaly in PTT testing; the aPL immunoassays were developed ~30 years ago and were derived from the biologic false positive syphilis test. Despite these empiric ori-

gins, the assays have proven useful in diagnosing patients at increased risk for recurrent thrombotic events. There are, however, significant limitations. For example, these assays cannot be used to diagnose APS until after a first, and possibly devastating, clinical event. Also, the individual aPL assays frequently do not correlate with other assays in the same patient, creating doubt as to the true diagnosis. Finally, there are patients who appear to have clinical manifestations of APS but test negative by all of the conventional aPL assays; these patients may have a seronegative form of APS (SNAPS), an entity that cannot be proven but may require long term anticoagulant therapy.

One of the main goals of our laboratory has been to develop assays that directly measure and report on putative APS mechanisms in patients' blood samples. An example of this approach is an assay that measures aPL-antibody-mediated resistance to annexin A5 anticoagulant activity. Annexin A5 is a natural anticoagulant which forms 2-dimensional crystals over membranes that express phosphatidylserine; aPL immune complexes create defects in the ordered crystallization of this protein that expose phosphatidylserine and thereby reduce the anticoagulant effect of this protein. This Symposium Abstract Lecture will review this approach along with others that have the potential of moving clinical assays for APS to quantifiable mechanistic bases. Mechanistic assays may improve diagnosis and lay the groundwork for novel non-anticoagulant treatments that target earlier steps in the disease process.

Disclosure of Interest: None declared.

AS034

Increased mortality in patients with the lupus anticoagulant: the Vienna lupus anticoagulant and thrombosis study (LATS)

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Background: Data on the clinical course of lupus anticoagulant (LA) positive individuals with or without thrombotic manifestations or pregnancy complications are limited.

Aims: To investigate mortality rates and factors that might influence mortality, we conducted a prospective observational study of LA positive individuals.

Methods: Starting from May 2001, adult patients that were repeatedly positive for LA according to current recommendations were consecutively included in this prospective study after having given written informed consent. The Ethics Committee of the Medical University of Vienna in accordance with the Declaration of Helsinki approved the conduct of the study [EC No. 068/2001].

Results: In total, 151 LA positive patients (82% female) were followed for median of 8.2 years. Thirty patients (20%) developed 32 thromboembolic events and 20 patients (13%) died. In univariable analysis, age (HR per year = 1.06, 95% CI 1.04–1.09, $P < 0.001$), hypertension (HR = 7.64, 95% CI 2.91–20.07, $P < 0.001$) and new onset of thrombosis (HR = 8.71, 95% CI 3.44–22.04, $P < 0.001$) were associated with an increased risk for death. New onset of thrombosis remained an adverse prognostic factor after multivariable adjustment for age and hypertension (HR = 6.26, 95% CI 2.57–15.25, $P < 0.001$). Concomitant autoimmune diseases, anticoagulant treatment at baseline or positivity for anticardiolipin- or anti- β_2 -glycoprotein I antibodies were not associated with mortality. In a relative survival analysis, our cohort of LA positive individuals showed a persistently worse survival in comparison to an age-, sex-, and study-inclusion-year-matched Austrian reference population. The cumulative relative survival was 95.0% (95% CI 88.5–98.8) after 5 and 79.7% (95% CI 69.2–87.0) after 10 years.

Conclusion: We conclude that occurrence of a thrombotic event is associated with higher mortality in patients with LA. Consequently, the prevention of thromboembolic events in LA positives might improve survival.

Disclosure of Interest: None declared.

AS035

Lupus anticoagulant activity can be explained by interference of anti- β 2GPI antibodies with the prothrombinase complex

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Background: Lupus anticoagulant (LA) is the most common acquired risk factor for thrombosis and is characterized by a prolongation of clotting times by autoantibodies against β 2-Glycoprotein I (β 2GPI). The general consensus is that anti- β 2GPI antibodies prolong clotting times by competing with coagulation factors for anionic phospholipids (PL). However, whereas addition of hexagonal (II) phase phospholipids attenuates the effects of anti- β 2GPI antibodies on clotting, these phospholipids do not support coagulation.

Aims: To reinvestigate how anti- β 2GPI antibodies prolong clotting *in vitro*.

Methods: We analyzed the effects of monoclonal anti- β 2GPI antibody 3B7 on coagulation with purified coagulation factors in normal and coagulation factor (F)V deficient plasma. Effects of 3B7 on the prothrombinase complex were determined with purified coagulation factors.

Results: FXa-initiated clotting times were 1.6-fold prolonged in plasma containing 3B7. Addition of an excess of PL attenuated the effects of 3B7, confirming LA activity. In contrast, 3B7 had no effect when clotting was initiated with both FVa and FXa, suggesting interference with assembly of the prothrombinase complex. Complexes of 3B7 and β 2GPI inhibited the activation of FV by FXa by 50% when the PL concentration was limiting, but not in the presence of excess PL. Addition of 3B7 to FV-depleted plasma supplemented with either FV or FVa prolonged FXa-initiated clotting times. However, prolonged clotting times were only corrected by excess PL in FV-depleted plasma supplemented with FV. Further analysis of prothrombinase reaction rates showed that 3B7 lowered the Vmax independent of the PL concentration. Human monoclonal anti- β 2GPI antibodies BK117 and EM6 showed similar results.

Conclusion: Anti- β 2GPI antibodies prolong clotting times in two ways. They inhibit the activation of FV by FXa in a PL-dependent manner and decrease the efficiency of the prothrombinase complex in a PL-independent manner.

Disclosure of Interest: None declared.

AS036

Circulating endothelial cells identify patients with antiphospholipid-antibodies and associated auto-immune diseases at risk for thrombosis

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Background: Endothelium damage has been described in antiphospholipid antibodies positive (aPL)-patients. However it is uncertain

whether circulating endothelial cells (CECs) – which are released when endothelial injury occurs – can identify patients at high risk of thrombosis.

Aims: To determine if CECs are associated with thromboembolic history in patients with auto-immune diseases.

Methods: This study included consecutive patients with aPL or Systemic Lupus Erythematosus (SLE). Blood was drawn at inclusion for aPL assays and CECs were assessed by CellSearch®, Veridex (CECs phenotype: CD146⁺, CD105⁺, DAPI⁺, CD45⁻). Bivariate and multivariate regression determined clinical and laboratory variables significantly associated with elevated CECs (> 20 mL⁻¹). Informed consent was obtained from each patient.

Results: Ninety-seven patients were included (mean age 46 $\pm$ 13 years, 77 women). Thirty seven patients had SLE and aPL and 60 only aPL. Eighty-one patients had a history of one or several thrombotic/obstetrical manifestations. In bivariate analysis, elevated CECs were significantly associated with history of myocardial infarction (Odds Ratio [OR] = 5.8 [95% CI 1.4–24.4], P = 0.02), pulmonary embolism (OR = 6.3 [95% CI 1.8–22.7], P = 0.006), small vessel thrombosis (OR = 3.6 [95% CI 1–12.3], P = 0.03), obesity (OR = 7 [95% CI 2–24.4], P = 0.003), and carotid plaque (OR = 4.3 [95% CI 1.2–14.7], P = 0.02). However, SLE (OR = 3 [95% CI 0.9–10.1], P = 0.07), and any aPL including triple positivity (OR = 0.6 [95% CI 0.2–2], P = 0.18) failed to reach statistical significance. In multivariate analysis, arterial (OR = 5.5 [95% CI 1.1–26.6], P = 0.03) and venous thrombosis (OR = 7.8 [95% CI 1.6–39.1], P = 0.01) remained significantly associated with elevated CECs.

Conclusion: This study demonstrates that endothelial injury assessed by CECs can identify patients with aPL and auto-immune diseases at risk for both arterial and venous thrombosis.

Disclosure of Interest: None declared.

AS037

Annexin A5 resistance correlates with multipositivity for antiphospholipid assays and is a prospective biomarker for adverse events

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Background: Currently, APS patients cannot be diagnosed until they have had at least one clinical event. In previous studies, the annexin A5 resistance (A5R) assay retrospectively identified antiphospholipid antibody positive patients with a propensity for thrombosis or pregnancy complications.

Aims: Since the probability of future adverse clinical events in asymptomatic patients is increased in the presence of multipositivity for APS criteria assays (LA, anti- β 2GPI antibody, and aCL antibody), we compared the levels of A5R to presence of multipositivity and to clinical outcomes.

Methods: We obtained samples from 121 patients tested for APS criteria assays and whose clinical histories were reviewed (RETROSPECTIVE cohort) and baseline samples from 71 asymptomatic patients who were observed over the course of 10 years (PROSPECTIVE cohort). All samples were tested for all three APS criteria assays and for A5R.

Results: In both retrospective and prospective cohorts, A5R values progressively decreased as the number of positive APS assays increased. Patients positive for all three APS criteria assays (i.e. triple positive) had significantly lower A5R values (mean $\pm$ SD: 166 $\pm$ 55%, n = 55) than patients with single positivity (mean $\pm$ SD: 221 $\pm$ 51, n = 60, P < 0.05) and healthy controls (mean $\pm$ SD:

271 ± 53, $n = 35$, $P < 0.001$). In the prospective cohort, 97% (30/31) of the triple positive asymptomatic patients developed an APS associated clinical event of which 80% (24/30) had a low A5R value of < 191% (i.e. < 2SD) vs. 40% (4/10) of the single positive patients developed an APS associated clinical event of which only 25% (1/4) had a low A5R value ($P = 0.17$, OR: 16). None of the healthy controls (0/20) had a low A5R value of < 191%.

Conclusion: Increased number of positive APS criteria assays was associated with reduced A5R values in the retrospective and prospective cohorts. A5R may identify patients who are at increased thrombotic risk and who may be candidates for treatment before a first thrombotic event.

Disclosure of Interest: None declared.

Microparticles

AS038

Microparticles in health and disease

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Extracellular vesicles (EV), including microparticles, are widely distributed. EV are present in liquids such as body fluids, ocean water, and beer. These EV originate from both eukaryotic and prokaryotic cells.

Over > 90% of EV in human body fluids have a diameter < 100 nm, and the total population provides a large surface area but a small volume. The main functions of EV are protection (coagulation, inflammation) and the transfer of biomolecules between cells. The concentration, cellular origin, composition and function of EV change in most if not all diseases, including cancer, cardiovascular disease, etc. This knowledge has raised great scientific and clinical interest, because vesicles potentially behold an entirely new level of clinically relevant information. Furthermore, changes in EV composition contribute to disease development, e.g. bleeding and thrombosis are now associated with the presence of EV exposing coagulant tissue factor in blood.

At present, isolation and detection of EV have become a 'hot topic'. Recently, we (re)introduced size-exclusion chromatography to isolate EV from body fluids such as plasma. So far, this methodology is very promising and has several important advantages compared to other methods. With regard to detection, to extract relevant clinical information such as cellular origin from single EV, ideally every single EV should be detected. Clearly, this is a huge challenge and 'work in progress' because most EV are extremely small, heterogeneous in size and shape, contaminants are present in the size range of EV, the refractive index of EV is low, 'swarm detection' may occur, etc.

Taken together, the presence and relevance of EV has now been firmly established. The next steps will be to further standardize pre-analytical conditions, to develop novel and reliable detection platforms for measurement of single EV, and to standardize functional assays.

Disclosure of Interest: None declared.

AS039

Microparticles from patients with acute coronary syndrome and heart transplanted patients induce premature endothelial senescence and thrombogenicity: role of oxidative stress and of the local angiotensin system

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Background: Microparticles (MPs) are surrogate markers of endothelial injury and cardiovascular risk. They circulate at high concentrations during acute coronary syndrome (ACS) and heart transplant rejection (HT), bear tissue factor (TF) and behave as prothrombotic effectors. Replicative endothelial senescence is associated with telomere shortening and MP generation in cultured endothelial cells (ECs).

Aims: To study the effects of circulating MPs from ACS and HT patients on the development of premature endothelial senescence and thrombogenicity.

Methods: MPs were isolated from venous blood of patients with ACS (6) or HT (6), and from 6 healthy volunteers (HV). MPs were applied to young first passage ECs from porcine coronary artery. EC senescence was assessed by flow cytometry using C12FDG, a probe for senescence-associated β -galactosidase activity, and the level of markers of oxidative stress (NADPH oxidase subunits), thrombogenicity (tissue factor, TF) and of cell cycle arrest by Western blot. In addition senescence markers were also assessed in atherosclerotic plaques of ACS patients, and in mammary arteries of patients with cardiac bypass surgery, according to risk factors.

Results: Compared to HV, circulating MPs from ACS and HT prompted premature senescence in young ECs and up-regulated the expression of NADPH oxidase (gp91phox, p47phox, p22phox), COX-2, senescent markers p53, p21, p16, actors of the local angiotensin system (AT1 angiotensin receptor, angiotensin converting enzyme) and of TF. MPs also down-regulated eNO synthase. Losartan, an AT1 receptor antagonist, prevented MPs-induced premature endothelial senescence. Senescence markers in plaques and mammary arteries were up-regulated when the number of risk factors increased.

Conclusion: MPs isolated from ACS and HT patients induced premature endothelial senescence and thrombogenicity through enhanced oxidative stress. Moreover, these data shed light on the importance of the local angiotensin system in MPs-mediated induction of endothelial senescence.

Disclosure of Interest: None declared.

AS040

The Gas6-Axl interaction mediates endothelial uptake of platelet microparticles

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Background: Upon activation platelets release plasma-membrane derived microparticles (MPs), which expose phosphatidylserine (PS) on their surface. The function and physiological clearance mechanism

of these MPs are incompletely understood. As they are pro-coagulant and potentially pro-inflammatory, rapid clearance from the circulation is essential for prevention of thrombotic diseases. The tyrosine kinase receptors Tyro3, Axl and Mer (TAMs) and their ligands protein S and Gas6 are involved in the uptake of PS-exposing apoptotic cells in macrophages and dendritic cells. Both TAMs and their ligands are also expressed in the vasculature, the functional significance of which is poorly understood.

Aims: We aimed to investigate whether platelet MPs are taken up in human endothelial cells and if this process is dependent on TAM-receptors and their ligands.

Methods: Platelet MPs, generated by exposing purified human platelets to a calcium ionophore, were isolated by ultracentrifugation and labeled with biotin or PKH67. The uptake of labeled MPs in the presence of protein S and Gas6 in human aortic endothelial cells (HAEC), human umbilical vein endothelial cells (HUVEC) and the endothelial cell line EA.hy926 was monitored by flow cytometry, western blotting and electron microscopy. The involvement of each TAM-receptor in the phagocytic process was evaluated using TAM-blocking antibodies or siRNA knock-down of individual TAMs.

Results: Platelet MPs bound Gas6 and protein S. All three types of endothelium were found to ingest platelet MPs in a Gas6 and Axl-dependent process. In contrast, protein S did not stimulate the uptake of MPs.

Conclusion: This study presents a new role for Gas6 and endothelial Axl beyond regulating cell survival and endothelial-monocyte adhesion. Uptake of platelet MPs in endothelium may promote rapid clearance of released MPs and provide a mechanism for platelet-endothelial communication and transportation of MPs from the circulation into the tissue.

Disclosure of Interest: None declared.

AS041

Platelets release extracellular vesicles in an agonist dependent manner but release a consistent profile of microrna

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Background: Platelets contain abundant microRNAs which can be packaged into extracellular Vesicles (EV) and released into the circulation. Platelets release two types of EVs; procoagulant microparticles (MP) and exosomes.

Aims: We characterised the phenotype and miRNA content of EVs released from platelets stimulated by different agonists.

Methods: Washed platelets from healthy subjects were maximally stimulated with agonists specific for GPVI (CRP-XL), PAR1 (SFFLRN), PAR4 (AYGPKF), P2Y1/P2Y12 (ADP) or PAR1/PAR4 (thrombin), with/wo inhibiting COX-1 (aspirin), aggregation (GPIIb-IIIa mAb) or ADP (apyrase). Released EVs isolated by differential centrifugation were characterised by size (Nanosight) and for procoagulant activity (Annexin-V binding and Stago CAT assay), or the exosome-specific markers CD63 & HSP70. RNA was isolated from EV populations, reverse-transcribed and amplified, and the miRNA profiled on Taq-Man microRNA microarray cards.

Results: Stimulation through GPVI produced a mixed population of MPs and exosomes, while all other agonists released predominantly exosomes. Of the inhibitors, only apyrase reduced EV release for all agonists. The EVs contained between 57 and 79 different miRNA with a core of 36 miRNA observed with all agonists. There is high correlation of agonist profiles ($r^2 > 0.98$ for all), and also with the total platelet miRNA content ($r^2 > 0.98$).

The 36 miRNA seen in all samples are predicted to have significant effects on the translation of proteins involved in endocytosis, cell cycle

control and differentiation. miR-223 was most highly expressed in all samples and has previously been shown to affect myeloid lineage development and have anti-inflammatory and cardioprotective effects.

Conclusion: These data suggest that while the EV profile released from platelets is agonist-dependent, all agonists release exosomes with similar miRNA content. ADP plays an important role in the release of exosomes. The data also suggest exosomes as the most likely vehicle for miRNA release from platelets.

Disclosure of Interest: None declared.

AS042

A prospective evaluation of the prothrombotic and prognostic potential of circulating microparticles in glioblastoma multiforme(GBM), pancreatic adenocarcinoma (PAC) and esophageal-gastric cancer (EGC)

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Background: At present is unknown if microparticles (MP) can be considered as prothrombotic and prognostic biomarkers in cancer.

Aims: To evaluate the predictive value of MP in venous thromboembolic events (VTE) and disease progression in glioblastoma multiforme (GBM), pancreatic (PAC) and esophageal-gastric (EGC) cancer.

Methods: MP level and activity was evaluated in samples at baseline, 1, 3, 6 and 12 months post diagnosis in 90 newly diagnosed patients (30 from each with GBM, PAC and EGC) and 90 sex and age-matched healthy controls. Informed consent obtained from all subjects. MP were identified by size and annexin V-FITC labelling using flow-cytometry. MP type were identified with specific antibody markers: CD62P for platelet-derived MP, CD62E for endothelial MP, CD45 for leukocyte MP, CD142 for tissue factor-bearing (TF+) MP, anti-gliial fibrillary acidic protein (GFAP)-FITC in GBM, CD227-FITC for mucin-bound (MUC-1 + MP) in PAC and CD326-APC for Ep-CAM + MP in EGC. Procoagulant MP activity was measured using the STA[®] Procoag PPL assay and by thrombin generation (TG).

Results: All cancer patients had significantly higher levels of total MP (measured as median and interquartile range), platelet MP, endothelial MP, leukocyte MP and TF + MP than controls ($P < 0.01$ for all). TG and PPL activity were similarly elevated ($P < 0.01$). There was a progressive increase in all MP subtypes with time and this increase became statistically significant at both 6 and 12 months compared with basal levels. Patients with GBM and PAC had significantly elevated tumour-derived MP. Patients with elevated TF + MP were at higher risk of VTE: hazard ratio (HR): 7.1 [1.18–33.25] for GBM and 4.6 [1.2–46] for PAC. Raised MUC-1 was associated with a bad prognosis in PAC (HR 3.1 [1.2–9.1]). No VTE or disease progression occurred in EGC group.

Conclusion: Basal levels of TF+MP predict the subsequent development of VTE in patients with GBM and PAC. High MUC-1-MP may be a predictive marker of poor prognosis in PAC.

Disclosure of Interest: E. Campello, C. Radu, L. Spiezia, S. Gavasso and P. Simioni: None declared; B. Woodhams Shareholder of: Haemacon Ltd.

Obesity – Bench to bedside

AS043

Endothelial cells and cross-talk with the adipocyte in the context of obesity

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White adipose tissue (WAT) is the main energy storage organ in mammals producing a large panel of molecules such as adipokines involved in energy/vascular homeostasis. During obesity, WAT expansion, particularly visceral depot (VAT), is associated with chronic inflammation, vascular alterations and predisposition to metabolic and cardio-vascular complications such as type 2 diabetes. In particular, endothelial cells (ECs) from VAT exhibit a proinflammatory and senescent phenotype.

With the aim to determine the contribution of ECs to adipocyte dysfunction, we performed co-culture experiments in a 3D setting associating human mature adipocytes and ECs, isolated from obese VAT. Co-culture experiments highlighted an inflammatory cross-talk leading to metabolic and secretory alterations of adipocyte functions with a decrease in the lipolytic activity, adipokine secretion, and insulin sensitivity conversely with increased production of several inflammatory molecules such as Interleukin (IL)-6, G-CSF and chemokines CXCLs. IL-6 and IL-1 β were identified as potential actors in these adipocyte alterations. The inflammatory burst was not observed in co-cultured cells from lean subjects. Interestingly, pericytes, in functional interactions with ECs, exhibited a proinflammatory phenotype with diminished angiopoietin-1 (Ang-1) secretion in WAT from obese subjects. Using the anti-inflammatory Ang-1, we corrected some deleterious effects of VAT-ECs on adipocytes, improving lipolytic activity and insulin sensitivity and reducing the secretion of proinflammatory molecules. Inflammation in adipocytes/ECs co-cultures was also reduced when functional pericytes (i.e. non inflammatory, producing Ang-1) were added to the co-cultures.

In conclusion, we identified a negative impact of VAT derived ECs on adipocyte functions during human obesity. Therapeutic options targeting EC inflammation could prevent adipocyte alterations that contribute to obesity comorbidities.

Disclosure of Interest: None declared.

AS044

Silencing of SEC23A and flotilin protects endothelial cells from developing a proinflammatory/prothrombotic phenotype in obesity

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Background: Adipokines are thought to promote atherothrombosis. In this regard, adipokines present in the secretome from the adipose tissue

of obese individuals induce the expression of a proinflammatory and prothrombotic phenotype in the endothelium.

Aims: The present study was carried out to identify the molecules differentially expressed in endothelial cells exposed to the secretome and to evaluate their impact on endothelial damage.

Methods: Omental adipose tissue was obtained from morbid (BMI: 41–43) obese (OB) females ($n = 8$), without other cardiometabolic risk factors; and from normoweight (BMI: 21–24) matched controls (C). Fat pads were incubated with nonfetal medium (24 h) to obtain visceral fat pad conditioned medium (VCM). Human endothelial cells (EC) were grown with medium containing 10% VCM. After 7 days, proteomic analysis was performed. Differentially expressed proteins were selected, checked by Western-blot, and specific siRNA were designed. After transfection, EC were grown in presence of medium with 10% VCM to evaluate expression of adhesion molecules (VCAM-1, ICAM-1) and extracellular matrix VWF.

Results: Seventy-four proteins were differentially expressed in EC exposed to OB-VCM vs. C-VCM. Silencing of the selected proteins Sec23A ($n = 6$) and flotilin ($n = 6$) resulted in a decreased expression of VCAM-1 ($56 \pm 11\%$ and $79 \pm 23\%$, respectively), ICAM-1 ($37 \pm 9\%$ and $66 \pm 10\%$, respectively) and VWF ($47 \pm 24\%$ and $59 \pm 34\%$ respectively) (values expressed vs. 100% expression in non-silenced cells). Silencing of annexin1 ($n = 6$) increased the proinflammatory insult induced by OB-VCM ($123 \pm 19\%$, $123 \pm 15\%$, $61 \pm 18\%$ for VCAM-1, ICAM-1 and VWF).

Conclusion: Exposure to the secretome of visceral adipose tissue induces changes in the proteome of endothelial cells, promoting a proinflammatory and a prothrombotic phenotype. Sec23, flotilin and annexin 1 are potential inducers of these changes. Identification of new markers of endothelial damage in obesity is ongoing (SAF2011-28214, RETIC/FEDER-RD12/0042/0016, FIS-PI13/00517).

Disclosure of Interest: None declared.

AS045

DNA methylation profiling identifies a locus near a HMG-CoA synthase gene associated with triglyceride levels and modified by BMI

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Background: In a candidate gene study using biobanked whole blood DNA, we reported association of DNA methylation marks (DNAm) in the *CTPIA* gene with triglyceride (TG) levels in families and cases with venous thromboembolism (VTE). The *CTPIA* gene codes for a protein expressed in liver and essential for fatty acid oxidation, thus supporting the role of DNAm in TG regulation.

Aims: To explore the role of epigenetic marks in the inter-individual variation of fasting TG levels.

Methods: We conducted a genome-wide investigation of blood DNAm in 214 individuals from the French-Canadian Family study on F5L Thrombophilia (F5L), a study sample composed of 5 large families ascertained on single probands with VTE. Replication was conducted in 350 unrelated VTE cases from the MARseilleTHrombosis Association Study (MARTHA). We used linear mixed regression models adjusted for age, sex and cell type proportions; and for familial corre-

lation in the *F5L* study. Sensitivity analyses taking into account potential confounders and effect modifiers (BMI, smoking, oral contraceptive) were conducted.

Results: We replicated the recently reported DNAm-TG association in *ABCG1*, a lipid-related gene, and discovered a novel genome-wide significant association in the *PHGDH* gene. The effect size at the *PHGDH* locus was stronger in the F5L study ($\beta = -0.2$; $P = 1.2 \times 10^{-6}$) than in MARTHA ($\beta = -0.08$; $P = 0.04$). The prevalence of overweight individuals ($\text{BMI} \geq 25$) was 56% and 35%, respectively, in the F5L and MARTHA studies. A stratified analysis in MARTHA revealed BMI being an effect modifier, with effect detected in overweight individuals only ($\beta = -0.2$, $P = 0.008$ vs. normal BMI $\beta = 0.02$, $P = 0.73$).

Conclusion: The DNAm mark found in *PHGDH* is only 34 kb from *HMGCS2*, a gene with similar function as *CTPIA*. *HMGCS2* belongs to the HMG-CoA synthase family known for its pivotal role in cholesterol synthesis and ketogenesis. These findings may shed light on the molecular mechanisms underlying the VTE-obesity association.

Disclosure of Interest: None declared.

AS046

Obesity measures, CRP and risk of venous thromboembolism and myocardial infarction

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Background: Low-grade inflammation is an apparent feature in obesity known to play an essential role for the relation between obesity and atherosclerosis-associated diseases. Thus, it is likely to assume that obesity-derived inflammation may be a shared pathway for the risk of venous thromboembolism (VTE) and myocardial infarction (MI).

Aims: To investigate the impact of C-reactive protein (CRP) on risk of MI and VTE in normal-weight and obese subjects recruited from a general population.

Methods: CRP and obesity measures were collected from 5995 subjects aged 25–84 years, who participated in the Tromsø Study in 1994–95. Incident VTEs and MIs were registered until January 1, 2011. Cox-proportional regression models were used to calculate hazard ratios of MI and VTE by different categories of CRP and obesity measures.

Results: There were 268 VTEs and 850 MIs during a median follow-up of 15.7 years. Waist-height ratio (WtHR) and waist circumference (WC) showed the highest correlations with CRP (Pearson's r 0.30 and 0.27, respectively). High levels of CRP ($> 3 \text{ mg L}^{-1}$ vs. $< 1 \text{ mg L}^{-1}$) were associated with a significant 50–80% increased risk of MI in all categories of WC and WtHR.

The joint effect of high WtHR or WC and CRP on risk of MI was slightly stronger than the sum of the individual components alone (synergy indices 1.30 and 1.16). Contrary, CRP levels were not associated with risk of VTE in any WtHR or WC category, and no synergistic effect was found. Risk estimates for MI in the highest vs. the lowest quintiles of the obesity measures were attenuated by 17–43% after adjustment for CRP, while the corresponding risk estimates for VTE were not affected by adjustment for CRP.

Conclusion: CRP was associated with increased risk of MI, but not with VTE in both normal-weight and obese individuals. Our findings suggest that low-grade inflammation does not play a key role in the causal pathway of obesity-related VTE.

Disclosure of Interest: None declared.

AS047

Visceral adiposity independently determines thrombin generation lag time and peak thrombin in morbid obesity

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Background: Obesity is a modifiable risk factor for morbidity including venous thromboembolism and cardiovascular disease. The cardiovascular risk associated with obesity is influenced by fat distribution; however the effect of fat compartmentalisation on markers of hypercoagulability is unknown.

Aims: This study evaluates the impact of abdominal fat compartmentalisation on hypercoagulability markers and thrombin generation parameters in grade III obese individuals.

Methods: Morbidly obese individuals attending the obesity clinic at King's College Hospital were consecutively recruited. Blood was taken for markers of hypercoagulability including D-dimer (DD), Factor VIII, fibrinogen and thrombin generation measured with calibrated automated thrombography. Single slice CT scans at L4 were performed to measure visceral (VAT) and subcutaneous adipose tissue (SAT).

Results: Eighty nine subjects were recruited into the study. The following parameters were elevated in subjects: DD, fibrinogen, factor VIII, factor VII, plasminogen activator inhibitor, tissue factor pathway inhibitor (TFPI) and thrombin generation; lag time, peak and endogenous thrombin potential. VAT correlated positively with FVIII, fibrinogen, free protein S, TFPI, thrombin generation lag time, peak thrombin, thrombin generation time to peak. VAT was also negatively correlated with antithrombin (Pearson's $r = -0.275$). There was no relationship between SAT and any of the haemostatic markers. Using multiple regression analysis and adjusting for age, sex and ethnicity, VAT was found to be an independent determinant for peak thrombin and thrombin generation lag time (standardised regression coefficients: 0.378 and 0.308).

Conclusion: VAT is an independent determinant of thrombin peak in class III obesity. In contrast, SAT was not associated with hypercoagulability. This suggests a possible role for VAT via adipocytokines in mediating the hypercoagulable state in morbid obesity.

Disclosure of Interest: None declared.

Sickle cell disease

AS049

Kininogen deficiency attenuates thrombin generation in a mouse model of sickle cell disease

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Background: We recently showed that tissue factor (TF) expressed by leukocytes activates coagulation in mouse model of sickle cell disease (SCD) and via microthrombotic events contributes to local lung inflammation, whereas endothelial cell TF does not contribute to coagulation but mediates factor Xa (FXa)-PAR2 signaling that enhances IL-6 expression. Treatment with FXa inhibitor rivaroxaban attenuated

both thrombin generation and IL-6 expression in sickle cell mice. Clinical data suggest that intrinsic coagulation pathway and kallikrein/kininogen system may be activated in SCD patients.

Aims: We investigated if these two pathways contribute to the hypercoagulable state and IL-6 expression in sickle mice (Townes model).

Methods: Bone marrow from sickle (S) and non-sickle (A) Townes mice, was transplanted into wild-type (WT), FXII^{-/-}, FXI^{-/-}, as well as high molecular weight kininogen (HK)^{+/+} or HK^{-/-} recipients. Mice were used for experiments 5 months after transplantation ($n = 10$ –24 mice).

Results: Thrombin generation, measured by plasma thrombin anti-thrombin (TAT) levels, was increased in WT mice injected with S bone marrow (WT/BM^S) compared to WT/BM^A mice (1.7-fold, $P < 0.05$), but neither FXII nor FXI deficiency reduce thrombin generation. In addition, inhibition of FXIIa-dependent activation of FXI with 14E11 antibody also did not reduce plasma TAT levels in SS mice. Importantly, increased plasma TAT levels in HK^{+/+}/BM^S (1.6-fold, $P < 0.01$ vs. HK^{+/+}/BM^A) were significantly decreased in HK^{-/-}/BM^S mice (4.8 ± 0.5 vs. 3.48 ± 0.2 ng mL⁻¹, $P < 0.05$). *In vitro*, HK fragments induce TF expression on monocytes. Increased plasma levels of IL-6 observed in WT/BM^S mice (5.6-fold, $P < 0.01$ vs. WT/BM^A) were not affected by any deficiency.

Conclusion: Our data indicate that HK, but not FXII and FXI, contributes to thrombin generation in SCD at steady state. Targeting HK fragments-induced TF expression on monocytes may be a promising approach to reduce hypercoagulable state in SCD without impacting hemostasis.

Disclosure of Interest: None declared.

AS050

Microparticles generated during storage of packed red blood cell units support thrombin generation

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Background: Intact RBCs appear to support thrombin generation in *in vitro* models of blood coagulation. During storage of packed red blood cell (pRBC) units, biochemical, structural and physiological changes occur including alterations to RBC membranes and release of microparticles.

Aims: The goal of this study was to assess the formation of procoagulant microparticles over the normal storage period of pRBC units.

Methods: On days 7, 14, 21, 28, 35 and 42 post production, five leukocyte-reduced pRBC units were sampled and aliquots of cells processed by differential centrifugation to provide washed cell and microparticle fractions. The ability of these fractions to support prothrombinase assembly and function was assessed. High resolution flow cytometry and scanning electron microscopy (SEM) were used to characterize the microparticle fraction.

Results: The rate of thrombin formation by microparticle isolates linearly increased ~8 fold from day 7 to day 42 of pRBC storage ($n = 5$), with the microparticle fraction accounting for the majority of the prothrombinase activity associated with pRBC units after 28 days. Flow cytometric analysis of microparticle isolates performed using anti-glycophorin A-PE identified RBC-derived microparticles, all of which also expressed phosphatidylserine as evidenced by binding of bovine lactadherin-FITC. SEM of a 42 day microparticle isolate showed the presence of microparticles ranging from 103 to 211 nm in diameter; in the washed RBC isolate, SEM showed the presence of similar sized vesicles extruding from the surface of RBCs.

Conclusion: Accumulation of RBC-derived vesicles capable of supporting prothrombinase function is a continuous process during storage. This suggests an increased potential of transfused units as they age to interact in unplanned ways with ongoing hemostatic processes in

injured individuals, especially given the standard blood bank practice of using the oldest units available.

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AS051

Specific inhibition of GPIIb/IIIa shedding improves post-transfusion recovery and adhesive function of outdated human apheresis platelets

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Background: Platelets (PLTs) undergo progressive and deleterious modifications during storage, an important feature of which is the ectodomain shedding of GPIIb/IIIa. Although inhibiting sheddase activity hampers clearance of PLTs with storage lesion, the role of GPIIb/IIIa shedding in PLT clearance is not defined. An anti-GPIIb/IIIa monoclonal antibody 5G6 has been developed to specifically inhibit GPIIb/IIIa shedding.

Aims: To test if 5G6 preserves quality and viability of stored human leukoreduced apheresis-derived PLTs.

Methods: The tight binding of 5G6 and its epitope in GPIIb/IIIa was studied by X-ray crystallography. Each of eight 2-day-old apheresis units was divided evenly and stored with 5G6 Fab fragment, control Fab or saline solutions under the standard blood bank condition for 8 days. Before and periodically during storage, aliquots were retrieved from the storage bag and analyzed for GPIIb/IIIa expression, PLT activity and adhesion under flow. Post-transfusion survival of stored PLTs in SCID mice was tracked by flow cytometry.

Results: The 5G6 Fab-epitope complex structure was solved to 2.4-Å resolution. It explains the binding specificity of 5G6 to the GPIIb/IIIa shedding cleavage site. 5G6 Fab treatment inhibited GPIIb/IIIa shedding in apheresis PLTs during storage. Compared with control PLTs, 5G6 Fab-stored PLTs exhibited the same levels of pH, PS exposure, PAC-1 binding, P-selectin expression, and agonist-induced aggregation. 5G6 Fab-stored 8-day-old PLTs formed significantly more and larger thrombi over the collagen surface under flow than control 8-day-old PLTs. The post-transfusion recovery of 5G6 Fab-stored 8-day-old PLTs in SCID mice was markedly higher than that of control 8-day-old PLTs and statistically indistinguishable from that of fresh 2-day-old PLTs.

Conclusion: Specific inhibition of GPIIb/IIIa shedding during storage improves post-transfusion recovery and adhesive function of outdated human apheresis PLTs, providing clear evidence for GPIIb/IIIa shedding as a cause of PLT clearance.

Disclosure of Interest: None declared.

AS052

The global impact of pathogen reduction systems on the platelet mRNA transcriptome, analysed by differential expression of RNA-Seq data

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Background: Platelet concentrates (PCs) are prepared at blood banks for patient transfusion in certain clinical conditions, associated with a low platelet count. To prevent transfusion-transmitted infections via PCs, different pathogen reduction (PR) systems have been developed that inactivate the nucleic acids of contaminating pathogens by chemical cross-linking, a mechanism that may also affect platelets' nucleic acids. We previously reported that treatment of stored platelets with the PR system Intercept significantly reduced the level of 6 microRNAs, induced platelet activation and compromised the platelet response to physiological agonists.

Aims: These observations prompted us to investigate the global impact of PR on the platelet mRNA transcriptome.

Methods: Genome-wide differential expression (DE) RNA sequencing (RNA-Seq) analysis by Next-Generation sequencing was applied to study the mRNA transcriptional landscape of platelets. One control group (untreated platelets) was compared with four platelet groups treated with common pathogen reduction systems.

Results: We report that Intercept markedly perturbs the mRNA transcriptome of human platelets and alter the expression levels of more than 1000 mRNAs ($P < 0.05$) compared to other PR systems and control platelets. Furthermore, integrated analysis combining expression data for microRNA (miRNA) and mRNA with prediction of miRNA-mRNA interactions disclosed several negative correlations between downregulated miRNAs and upregulated target mRNAs in Intercept-treated platelets.

Conclusion: In conclusion, this study demonstrates that Intercept markedly alters the platelet mRNA transcriptome by diminishing the expression of a significant number of genes and by elevating the expression levels of other mRNAs as a result of reduced expression levels of their regulatory miRNAs.

Disclosure of Interest: None declared.

Diagnostic techniques in atherothrombosis

AS054

Whole exome sequencing in patients with inherited thrombocytopenia and excessive bleeding is an efficient way to identify genetic variants in known and novel genes

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Background: Inherited thrombocytopenias are a heterogeneous group of disorders characterised by a low platelet count which is associated with abnormal bleeding. A normal platelet count in whole blood is $150\text{--}450 \times 10^9 \text{ L}^{-1}$ which is maintained within a narrow range via homeostatic processes. Next generation sequencing has previously been employed for the confirmation of suspected genetic abnormalities and more recently the discovery of novel disease causing genes but its full potential has not previously been utilised in a large patient cohort.

Aims: To determine the genetic aetiology of disease and novel variant discovery in a UK-wide patient cohort with clinical bleeding and a suspected inherited thrombocytopenia.

Methods: Patients are analysed using a unique approach combining platelet phenotyping, by flow cytometry, and genotyping, consisting of targeted panel and whole exome sequencing, as outlined by the UK-Genotyping and Phenotyping of Platelets (GAPP) study group. Whole blood cell counts are determined using the Sysmex XN-1000 haematology analyser (Whole blood platelet counts, MPV, IPF).

Results: Over the course of the past 6 years we have recruited over 600 patients with suspected platelet function disorders to the GAPP study. To date 52 patients, with platelet counts varying between $11\text{--}186 \times 10^9 \text{ L}^{-1}$, from 34 index cases have undergone whole exome sequencing. We have identified and confirmed the genetic basis of disease in 71% (24/34) of patients. Variations in the 29 known thrombocytopenia causing genes account for 19 patients (*ACTN1*, *ANKRD26*, *CYCS*, *FLI1*, *GFI1B*, *GP1BA*, *MKLI*, *MYH9*, *NBEAL2*, *RUNX1* and *TUBB1*). Five patients show novel genetic aetiology (*ANKRD18A*, *PF4* and *SLFN14*).

Conclusion: Whole exome sequencing, combined with platelet phenotyping, is an efficient method for determining the genetic cause of disease in patients with inherited thrombocytopenia. In addition it aids in the discovery of novel genes which can help further our knowledge of platelet biology.

Disclosure of Interest: None declared.

AS055

A novel platform to study the mechano-sensing mechanism on a single aspired platelet

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Background: Under conditions of rapid blood flow, adhesion and signaling of platelets are initiated by their surface receptors GPIIb α and integrin α _{IIb} β ₃ binding to sub-endothelial ligands: von Willebrand factor (VWF) and fibrinogen (Fg). Such interactions (VWF–GPIIb α and Fg– α _{IIb} β ₃) can trigger outside-in signals, characterized by distinct elevations of cytosolic calcium (Ca²⁺). However, the mechano-sensing mechanisms still remain obscure.

Aims: Armed with a novel single-molecule approach: fluorescence bio-membrane force probe (fBFP), we propose to elucidate how force triggers a single GPIIb α and α _{IIb} β ₃ bond mediated platelet signal.

Methods: In fBFP, we simultaneously measured and analyzed binding and intracellular Ca²⁺ of single platelets. VWF-A1 or Fg was coated on a bead attached to a red blood cell to form a force probe (left). A discoid human platelet pre-loaded with Fura2 was aspirated by an apposing micropipette to serve as a target (right). The platelet target repeatedly touched (mimics platelet translocation) the bead to probe each bond.

Results: Our experiment revealed that a universal correlation was observed between the pre-Ca²⁺ longest lifetime and the Ca²⁺ peak across wild type, 2B (R1450E) and 2M (V1279I) VWF-A1 mutants. This indicates a mechanoreception mechanism induced by a durable force on single GPIIb α and suggests von Willebrand disease may also be a signaling defect. Interestingly, the single Fg– α _{IIb} β ₃ bonds could also induce significant morphological structure that features body elongation, remodeling and contraction, which is similar to our recently characterized ‘tractopod’ structure – a branched platelet membrane anchor that is responsible for initiating platelet-endothelial interactions but insensitive to the classic anti-platelet treatments by aspirin and clopidogrel.

Conclusion: Single-molecule fluorescence technique fBFP provides the new molecular insights on how shear force regulates the platelet mechano-signaling and the consequences of their dysregulation in human blood diseases.

Disclosure of Interest: None declared.

AS056

Factor Xa overcomes inhibition by the direct factor Xa-inhibitors following insertion of a structural element from the serine protease domain of snake venom factor Xa

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Background: The direct factor Xa (FXa)-inhibitors apixaban and rivaroxaban are small compounds that tightly bind the FXa active site and effectively block all catalytic activity. Strikingly, the FXa homolog from the venom of the elapid snake *P. textilis* shows minimal sensitivity to inhibition by the FXa-inhibitors. This homolog contains a 13-residue insertion between His91-Tyr99 (chymotrypsin numbering) in the serine protease domain, which is conserved in venom-FXa-producing Australian elapids only. A recent crystal structure of a venom-FXa isoform shows this region to be in close proximity of the active site.

Aims: We hypothesize that this insertion accounts for the minimal sensitivity of *P. textilis* venom-FXa to the FXa-inhibitors.

Methods: To investigate this, we constructed and stably expressed three human FX variants (FX-A, FX-B, FX-C) that incorporate the insertion from different venom-FXa isoforms.

Results: Upon purification, RVV-X-activation, and isolation by size-exclusion chromatography, peptidyl substrate conversion by all FXa variants revealed an up to 500-fold reduced sensitivity towards the FXa-inhibitors. Moreover, once assembled into prothrombinase, all variants were able to sustain prothrombin conversion at significantly higher FXa-inhibitor concentrations (IC₅₀ 100–600 nM) compared to plasma-derived FXa (pdFXa; IC₅₀ 2 nM). FXa-initiated (5 nM) thrombin generation (TG) in human plasma demonstrated near normal TG profiles for the FXa variants. While apixaban (2 μ M) dramatically prolonged the lag time (32-fold) and reduced peak thrombin by 75% in pdFXa-initiated TG, these parameters were unperturbed with the FXa variants present.

Conclusion: In conclusion, insertion of venom-FXa regions into human FXa permits protease function in the presence of an excess of direct FXa-inhibitors. As such, these chimeric proteases have the potential to serve as rescue therapeutic agents to overcome the effect of FXa-inhibitors in case of potential life-threatening bleeding events or emergency surgical interventions.

Disclosure of Interest: D. Verhoef: None Declared, M. Schreuder: None Declared, C. van der Sluijs: None Declared, K. L. Cheung: None Declared, X. Yang: None Declared, P. Reitsma: None Declared, M. Bos Grant/Research Support from: Bayer Hemophilia Awards.

Protease activated receptors

AS057

Coagulation proteinases, proteinase-activated receptors and inflammation

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In addition to its ability to promote coagulation by converting fibrinogen to fibrin, thrombin is now known to regulate tissue function by cleaving and activating a unique set of proteolytically activated G-protein-coupled receptors (PARs 1 & 4). PAR signaling involves the cleavage-unmasking of an N-terminal receptor sequence that becomes a tethered ligand (TL). In addition to thrombin, Activated Protein-C (APC) also activates PAR1, but by cleaving at a distinct N-terminal site to generate a distinct ‘biased-signaling’ tethered ligand, TL-APC, that increases endothelial barrier function in contrast with the decrease caused by thrombin-PAR1 activation. Moreover, PAR1 signalling can involve its heterodimerization with PAR2. Coagulation and other site-specific proteinases can generate all of the classical hallmarks of acute and chronic inflammation (dolor, calor, rubor, tumor and functio laesa) by activating PARs on a variety of target cells ranging from sensory neurons to somatic parenchymal cells and the vascular endothelium. As will be discussed, these actions contribute to a variety of inflammatory diseases ranging from colitis, arthritis and multiple sclerosis to cancer. Thus, the PARs and their activating proteinases represent attractive therapeutic targets for treating inflammatory disease.

Disclosure of Interest: None declared.

AS058

Unravelling PAR1 signaling in endothelial cells using quantitative phosphoproteomicsvan den Eshof BL¹, van Alphen FP¹, Mertens K^{1,2}, Meijer AB^{1,2} and van den Biggelaar M¹¹Department of Plasma Proteins, Sanquin Research, Amsterdam;²Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands

Background: Thrombin-induced endothelial signaling has been recently dissected, revealing thousands of dynamically regulated phosphorylation sites. Proteinase-activated receptor 1 (PAR1) is the predominant thrombin receptor in endothelial cells (ECs), however it is unclear whether it accounts completely for this extensive phosphoregulation. This is particularly relevant, since PAR1 inhibitors have been developed as novel anti-platelet drugs and their effects on ECs are unclear.

Aims: The aim of this study is to dissect the contribution of PAR1, additional PARs and other receptors in thrombin signaling using an unbiased phosphoproteomic approach.

Methods: Blood outgrowth endothelial cells (BOECs) and platelets were stimulated with PAR1 peptide (10 μ M SFLLRN) or thrombin (1 U mL⁻¹) in presence/absence of PAR1 antagonist (100 nM Vorapaxar). Platelet aggregation and endothelial barrier function were measured using aggregometry and ECIS. Activation of p38MAPK was determined using a phospho specific antibody (T180/Y182). BOECs were metabolically labeled using SILAC and stimulated for 2/10 min in a triplicate labeling experiment as described above. Phosphorylated peptides were enriched using TiO2 precipitation, detected by Orbitrap Fusion Tribrid MS and analyzed using MaxQuant and Perseus software.

Results: In addition to inhibiting PAR1 mediated platelet aggregation, vorapaxar completely blocked thrombin- and PAR1-mediated p38 MAPK activation and endothelial barrier disruption. Principal component analysis of quantified phosphosites (> 3300) and hierarchical clustering of regulated phosphosites (> 200) showed that thrombin and PAR1 peptide induced identical phosphoregulation, which was completely blocked by vorapaxar.

Conclusion: This study indicates that only PAR1 mediates thrombin-induced phosphoregulation in ECs. In addition, PAR1-directed anti-platelet drugs have an unexpected direct side-effect on EC functioning. This approach will now be applied to further dissect signaling networks induced by different PAR1 agonists.

Disclosure of Interest: None declared.

AS059

Thrombin, stromal cell-associated PAR-1, and fibrin (ogen) promote colon cancer progressionAdams G¹, Rosenfeldt L¹, Frederick M¹, Miller W¹, Waltz D¹, Kombrinck K¹, Monia B², Revenko A² and Palumbo J¹¹Department of Hematology, Cincinnati Children's Hospital, Cincinnati, OH; ²Department of Antisense Drug Discovery, Isis Pharmaceuticals Inc., Carlsbad, CA, USA

Background: Hemostatic factors are powerful determinants of metastasis, but are generally dispensable for tumor growth. Several lines of evidence suggest that colon cancer represents at least one important exception.

Aims: To identify hemostatic factors which contribute to colon cancer progression.

Methods: We used gene-targeted mice to define the role of prothrombin, PAR-1, and fibrinogen in the growth and dissemination of colonic adenocarcinoma.

Results: Lowering circulating prothrombin levels to ~10% of normal in mice dramatically impeded the growth of murine- and human-derived colonic adenocarcinomas and nearly eliminated the metastatic potential of these cells. Colon cancer growth was also impeded in PAR-1^{-/-} mice, suggesting that thrombin drives colon cancer growth via activation of stromal cell-derived PAR-1. Furthermore, colon cancer growth was profoundly diminished in fibrinogen-deficient mice relative to control animals. Fibrinogen deficiency resulted in a 3-fold diminution in tumor mass over a 3 week study period. Tumor mitotic indices were 2-fold higher in tumors from control mice relative to those from fibrinogen-deficient mice. This finding was in stark contrast with previous studies where fibrinogen was dispensable for the robust growth of multiple non-gastrointestinal cancers. Fibrin crosslinking was not required for colonic adenocarcinoma proliferation, as tumor growth was unaffected by elimination of factor XIII. However, colon cancer growth was diminished in mice expressing a mutant fibrinogen lacking the α_{MB2} binding motif but maintaining normal clotting function, suggesting that fibrin(ogen)-leukocyte interactions promote the growth of colonic adenocarcinoma.

Conclusion: These data indicate that colon cancer represents one important malignancy where thrombin-mediated proteolysis drives both primary tumor growth and metastasis formation. These results also suggest that thrombin promotes colon cancer growth via mechanisms involving at least two distinct substrates, PAR-1 and fibrinogen.

Disclosure of Interest: None declared.

AS060

Tumor-derived tissue factor-expressing microvesicles promote a proadhesive phenotype in endothelial cellsChe S¹ and Stokol T²¹Biomedical Engineering; ²Cornell University, Ithaca, NY, USA

Background: Microvesicles (MV) are found in blood samples from human patients with cancer, with MV concentrations correlated to tumor malignancy. MV can promote tumor progression and metastasis by triggering signaling or transferring protein and RNAs to other (host or tumor) cells. Tissue factor (TF), over-expressed by most epithelial tumors, is found also found on tumor MV.

Aims: We hypothesized that TF-expressing tumor MV would activate endothelial cells via protease-activated receptors (PARs), upregulating adhesion molecules; such endothelial activation could potentially promote metastasis.

Methods: Tumor-conditioned media was collected from MDA-MB-231 and MCF-7, breast cancer cell lines that express high and low TF respectively. Media was ultracentrifuged at 100,000 $\times$ g for 70 min to collect MV. Human umbilical vein endothelial cells (HUVEC) were treated with MV with or without factor VIIa (FVIIa) and factor X (FX) for 6 or 24 h. We evaluated the expression of adhesion molecules (E-selectin, ICAM-1, and VCAM-1) using cell surface ELISAs.

Results: We found that MV derived from MDA-MB-231, but not MCF-7, induced upregulation of E-selectin and ICAM-1, but not VCAM-1, after 6 and 24 h of exposure. We observed higher adhesion molecule expression with increasing concentrations of FVIIa (0.1–10 nM) and FX (50–150 nM). The upregulation of adhesion molecules was abolished by a FXa inhibitor (FPRCK, 40 μ M) and an anti-TF antibody (HTF-1, 10 μ g mL⁻¹), but not hirudin (10 U mL⁻¹). Preliminary experiments with PAR-1/2 agonists and antagonists supported that the upregulation of surface adhesion molecules by TF-expressing MV was mediated through PAR receptors on HUVEC.

Conclusion: TF-expressing tumor MV can promote a proadhesive phenotype in endothelial cells, which can enhance metastasis by arresting tumor cells in circulation or recruiting hematopoietic-derived progenitor cells to form a pre-metastatic niche.

Disclosure of Interest: None declared.

VWF

AS061

The multiple roles of von Willebrand factor: regulation of angiogenesis

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The large multimeric glycoprotein Von Willebrand factor (VWF), best known for its role in haemostasis, is involved in multiple vascular processes. We recently described a new role for VWF in controlling angiogenesis. *In vivo*, using the VWF deficient mouse, we found increased angiogenesis in three different models, namely the Matrigel plug, the new-born retina angiogenesis and the hind limb ischemia models. *In vitro*, inhibition of VWF expression by siRNA resulted in increased endothelial cell migration, proliferation and angiogenesis; similar defects were found in endothelial cells differentiated *ex vivo* from blood outgrowth endothelial progenitor cells (BOEC) isolated from patients with Von Willebrand disease (VWD). These data indicate that endothelial VWF modulates the process of new vessel formation by exerting an inhibitory effect. VWF deficiency resulted in enhanced pro-angiogenic signalling through vascular endothelial growth factor receptor-2 (VEGFR2), one of the most potent activators of angiogenesis. Both intracellular and extracellular mechanisms have been implicated in the control of blood vessel formation by VWF, including angiopoietin-2 and integrin $\alpha v\beta 3$; their relative importance in modulating VEGFR2 signalling and angiogenesis is being investigated. These findings may have significant clinical implications for patients with VWD and angiodysplasia. Angiodysplasia has been linked to dysregulated angiogenesis, a condition of degenerative blood vessels present in the gastrointestinal tract which can cause severe intractable bleeding in patients with congenital VWD or in acquired conditions such as Heyde syndrome or left ventricular assisted device (LVAD).

Disclosure of Interest: None declared.

AS062

Von Willebrand factor is indispensable for venous hemostasis in a novel murine bleeding model

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Background: Von Willebrand factor (VWF) is crucial for arterial hemostasis, while its role in venous hemostasis is considered to be limited. The murine tail clip-bleeding model that is used to test hemostasis *in vivo* involves both arterial and venous vessels, and is thus unable to distinguish for the role of VWF in either hemostatic process.

Aims: To investigate the contribution of VWF to venous hemostasis in a novel tail-vein transection bleeding model.

Methods: Wild-type murine VWF (wt-mVWF), mVWF mutants or human FVIII were expressed in *Vwf*-deficient mice via hydrodynamic gene transfer and their effect on venous bleeding was assessed.

Results: Specific transection of the lateral tail vein in *Vwf*-deficient mice resulted in uninterrupted bleeding for 30 min with excessive blood loss (0.9 ± 0.3 mL; $n = 8$). Normalization of FVIII levels (1.6 ± 0.4 U mL⁻¹) via over-expression of wt-FVIII left the bleeding tendency unaffected (bleeding time ≥ 30 min; blood loss 0.7 ± 0.2 mL; $n = 8$; $P > 0.05$), indicating that bleeding is not due to reduced FVIII levels. Expression of wt-mVWF corrected the bleeding tendency (bleeding time: 2.2 ± 0.5 min ($n = 11$); blood loss: 0.2 ± 0.1 mL). No spontaneous rebleeding and limited rebleeding upon physical challenge was observed. Similar data were obtained for

a VWF mutant lacking collagen binding. Mice expressing VWF with defective binding to glycoprotein (Gp)IbIIIa (mVWF/p.D2509G) displayed a heterogeneous phenotype: 4 of 5 mice were similar to wt-mVWF, while one mouse displayed prolonged bleeding time (6.5 min), spontaneous rebleeds and increased blood loss (0.8 mL). Defective GpIb-binding (mutant mVWF/p.K1362A) induced an increased bleeding time (5 ± 2 min) and excessive rebleeds in 4 mice, while 2 additional mice bled non-stop for 30 min. Blood loss was significantly increased in all mVWF/K1562A-expressing mice (0.6 ± 0.2 mL; $n = 6$; $P < 0.02$).

Conclusion: Our data point to a previously unrecognized role for VWF-GpIb interactions in the arrest of venous bleedings.

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AS063

The introduction of a mucin insert at the N-terminus of VWF-A1, but not the C-terminus, enhances platelet GPIIb/IIIa binding to VWF

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Background: Von Willebrand Factor (VWF) A1-domain binding to the platelet receptor GPIIb/IIIa regulates thrombosis rates. While studies using different truncated VWF forms suggest that the domains and peptides flanking VWF-A1 control its binding to GPIIb/IIIa, this concept has not been tested using multimeric protein and *in vivo* models.

Aims: To determine if altering the physical distance between VWF-A1 and domains located at its N-terminus (i.e. VWF-D'D3) and C-terminus (VWF-A2) affect platelet-VWF interaction under shear.

Methods: A 20-amino acid mucin insert was introduced at either the N- or C-terminus of the A1-domain in two base constructs: multimeric human VWF (h-VWF) and a variant where the human A1-domain was replaced by murine-A1 (h[mA1]-VWF). Proteins were expressed in HEK cells or in *VWF*^{-/-} mice using hydrodynamic injection.

Results: In ELISA, insertion of the mucin at the N-, but not C-, terminus of human-A1 decreased the K_D of VWF-GPIIb/IIIa binding by 50–100 fold. In viscometer based shear induced platelet aggregation assays, also, VWF with the N-terminal mucin insert displayed a higher potential to trigger platelet activation and aggregation compared to h-VWF or the protein with the C-terminal insert. Additionally, the anti-D'D3 mAb DD3.1 inhibited platelet aggregation in runs containing h-VWF but not VWF with the N-terminal mucin. Similar to this, mucin insertion at the N- but not C-terminus of both h-VWF and h[mA1]-VWF dramatically decreased platelet translocation velocity, increased platelet accumulation and enhanced thrombus formation under fluid shear in studies performed using a microfluidic flow cell. Increased blood coagulation upon N-terminal mucin insertion was also noted in tail-bleed assays performed using *VWF*^{-/-} mice that expressed different h[mA1]-VWF variants.

Conclusion: Overall, the spacing between the VWF-D'D3 and A1-domain is a critical feature regulating the adhesive function of multimeric VWF both *in vitro* and *in vivo*. The physical distance between the A1 and A2-domains is less critical.

Disclosure of Interest: None declared.

AS064

High resolution microscopic characterisation of VWF biosynthesis, storage and secretion in type 1 VWD patients with large in-frame VWF deletions

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Background: Type 1 von Willebrand disease (VWD1) results from partial quantitative deficiency of von Willebrand factor (VWF) caused by mutations in the VWF gene (*VWF*). Previous studies identified three novel in-frame VWD1 deletions, involving exons 3 (VWFdel3), 32–34 (VWFdel32–34) and 33–34 (VWFdel33–34), with index cases presenting VWF:Ag levels of 31, 12 and 32 IU dL⁻¹ respectively and *in vitro* expression demonstrating reduced secretion of recombinant VWF (rVWF).

Aims: To use high resolution fluorescent microscopy to investigate biosynthesis, storage and secretion of 3 VWF deletion variants.

Methods: rVWF was transiently-transfected into HEK293 cells. 72 h post-transfection, cells were stained with immunofluorescent antibodies to VWF, endoplasmic reticulum (ER) and trans-Golgi network (TGN) enabling 3-channel co-localisation. Imaging used a Nikon TiE inverted deconvolution microscope and an OMX Structured Illumination Microscopy (SIM) system.

Results: WT rVWF transfections produced pseudo-Weibel-Palade bodies (WPB) at the TGN and in the cytoplasm. Homozygous (Hom) rVWFdel3 completely lacked WPB, with diffuse staining appearing to co-localise with ER as confirmed via SIM. Heterozygous (Het) rVWFdel3 produced small WPB-like structures and diffuse staining was reduced. Hom rVWFdel32–34 appeared to produce smaller, more rounded WPB than WT but larger WPB structures were visible in Het rVWFdel32–34. Hom rVWFdel33–34 produced small WPB-like structures that appeared to colocalise with TGN, whereas Het rVWFdel33–34 WPB were more dispersed and less TGN-associated.

Conclusion: Large exonic *VWF* deletions have profound effects on VWF maturation and WPB formation. Homozygous VWFdel3 abolishes WPB formation, possibly due to ER retention. VWFdel32–34 and VWFdel33–34 formed WPB-like structures but these were smaller and rounder than WT, suggesting defective VWF tubule arrangement during WPB formation in the Golgi. This study demonstrates that large exonic VWD1 deletions may cause reduced VWF secretion via defects in WPB biogenesis.

Disclosure of Interest: None declared.

AS065

The recognition of collagen by the VWF A domains

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Background: von Willebrand factor (VWF) has a crucial role in capturing platelets by subendothelial collagens that become exposed upon vascular injury or plaque rupture. The VWF A domains are central to this process, A3 binding to a known site in collagen, Lisman *et al.*, Blood, 108: 3753–3756 (2006), whilst A1 binds to platelet Glycoprotein Iba. Although a growing strand of literature reports that A1 may also bind collagen, this interaction remains ill-defined.

Aims: Identify the collagen motif(s) from Toolkit II and Toolkit III that involve VWF A1 binding.

Methods: We applied recombinant VWF A1 and A3 domains, expressed as either GST-fusion or His-tagged proteins, to the collagen Toolkits, libraries of synthetic triple-helical peptides, and measuring binding using a colorimetric static adhesion assay.

Results: As before, A3 bound to a single peptide in Toolkit III (representing the full COL domain of collagen III). Using Toolkit II, A3 bound the corresponding, conserved, site in collagen II. Unexpectedly, a second peptide in Toolkit II proved strongly A3-reactive, at a site conserved in collagen I but absent from collagen III. A1 domain was found to bind several conserved sites in both Toolkits, mapping its collagen-binding activity for the first time.

Conclusion: VWF can interact with collagen in two distinct ways, through both its A1 and A3 domains. The known A3 site in collagen II is not fully conserved in collagen I, being degenerate between its two α -chains, neither of which individually can bind A3: binding activity would require one specific interchain registration of collagen I. However, the new A3-binding site is conserved in both collagens I and II, and the novel A1 sites are conserved across all three collagens. We conclude that both the main fibrillar collagens of the vessel wall, I and III, can bind VWF via both its A1 and A3 domains. The relationships between these interactions remains to be established.

Disclosure of Interest: None declared.

Platelet signalling

AS066

Tyrosine phosphatases in platelet signal transduction

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Tyrosine phosphorylation is a primary mode of signal transduction regulating many aspects of platelet activation and thrombosis. Although much is known about the protein-tyrosine kinases (PTKs) that propagate activation signals in platelets, comparatively little is known about the protein-tyrosine phosphatases (PTPs) that modify these signals. Dephosphorylation of key tyrosine residues in proteins can alter enzymatic activity and disrupt protein-protein interactions. Ten receptor-like and 10 non-transmembrane PTPs have been identified in platelets by mass spectrometry; however, less than half have been validated and the functions of most remain undefined. A growing body of evidence has established that PTPs are just as important as PTKs in platelets, performing highly specialized functions, and acting as both positive and negative regulators of platelet activation. PTPs have also been implicated as critical regulators of megakaryocyte development and platelet homeostasis. Fully understanding how these processes are controlled will require an integrated comprehension of PTPs and PTKs, and how they work together to regulate the level of phosphorylation of specific sites in receptors and signalling proteins. This knowledge, together with recent advances in the development of increasingly specific PTP inhibitors may yield a new class of anti-platelet therapy, as well as novel strategies of controlling platelet production.

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Disclosure of Interest: None declared.

AS067

RUNX1 regulates megakaryocytic/platelet ras-related small gtpase RAB1B: potential role in platelet dysfunction with RUNX1 haploinsufficiency

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Background: Patients with RUNX1 haploinsufficiency have thrombocytopenia, abnormal platelet function and defective granule secretion.

Platelet expression studies in patient with thrombocytopenia, abnormal platelet aggregation and secretion, and RUNX1 mutation revealed downregulation of several genes including *ALOX12*, *MYL9*, *PRKCO*, *PF4* (Sun *et al.* 2007 JTH 5: 146–154). In addition, small RABGTPase *RAB1B* was significantly decreased relative to controls (fold change: 0.32, $P = 0.025$; confirmed by qPCR). RABGTPases play a major role in vesicle trafficking, secretion and granule targeting of proteins.

Aims: We addressed the hypothesis that megakaryocytic (MK)/platelet *RAB1B* is regulated by RUNX1 and alterations in *RAB1B* may contribute to platelet dysfunction.

Methods: *In silico* analysis of *RAB1B* promoter –1/–785 bp from the ATG revealed 4 RUNX1 consensus sites; site-1 (–440/–435), site-2 (–622/–617), site-3 (–660/–655) and site-4 (–770/–765). We performed studies in human erythroleukemia (HEL) cells treated with phorbol ester to induce MK transformation. We performed chromatin immunoprecipitation (ChIP) assay using HEL cell chromatin and electrophoretic mobility shift (EMSA) assays using nuclear protein to assess RUNX1 binding to each site. Luciferase-reporter studies were performed to assess promoter activity and the effect of RUNX1 overexpression and silencing.

Results: ChIP revealed RUNX1 binding to *RAB1B* sites-1 and -4. EMSA revealed RUNX1 binding to same sites. In promoter-reporter studies mutations of sites-1 and -4 individually reduced activity by 90%. RUNX1 overexpression enhanced *RAB1B* promoter activity by 2-fold and *RAB1B* protein ~30%. Enhancement of promoter activity was abrogated by mutations of sites-1 and -4 indicating that these sites were functional. RUNX1 siRNA reduced *RAB1B* promoter activity and *RAB1B* protein.

Conclusion: These results provide the first evidence that *RAB1B* is regulated by RUNX1. Alterations in *RAB1B* may be the basis of defective granules and secretion in RUNX1 haploinsufficiency.

Disclosure of Interest: None declared.

AS068

TRPM7 kinase regulates itam-signaling and plays a critical role in thrombosis and ischemic stroke

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Background: TRPM7 has been identified as a constitutively active cation channel which regulates Mg^{2+} and Ca^{2+} homeostasis in mammals. The C-terminus of TRPM7 contains an α -type Ser/Thr kinase domain. So far, only few substrates of TRPM7 kinase have been identified and the physiological function of the kinase activity remains elusive.

Aims: We aimed to elucidate the signaling function of TRPM7 kinase under normal and pathophysiological conditions.

Methods: A *Trpm7*^{KI} mouse line was generated by introducing a point mutation in the *Trpm7* gene leading to the complete loss of the kinase activity. Mutant platelets were analyzed using mass spectrometry, *in vitro* kinase assay, and a wide range of *in vitro* and *in vivo* functional assays.

Results: *Trpm7*^{KI} platelets display unaltered Mg^{2+} homeostasis. However, a markedly impaired IP_3 production, and consequently reduced Ca^{2+} mobilization upon stimulation of the ITAM-coupled collagen receptor GPVI was observed in *Trpm7*^{KI} platelets. Thapsigargin induced store operated calcium entry (SOCE) was also altered in *Trpm7*^{KI} platelets. Consequently, degranulation, integrin activation and aggregation responses to GPVI agonists were reduced. We investigated the phosphoproteome of GPVI-stimulated *Trpm7*^{KI} platelets to understand the molecular mechanism underlying these defects. Several hypo-phosphorylated proteins of the GPVI-LAT signalosome (Lyn, Syk, LAT, PLC γ 2) and the SOC complex (STIM1 and PKC ϵ) were identified. Furthermore, an *in vitro* kinase assay showed that TRPM7 kinase directly binds and phosphorylates Lyn, but not Syk. In line with the phosphorylation defects, *Trpm7*^{KI} mice were protected from arterial thrombosis and ischemic brain infarction.

Conclusion: These results establish the signaling function of TRPM7 kinase as a key regulator of ITAM-signal induced Ca^{2+} responses in platelets, and highlight the role of TRPM7 kinase in the pathogenesis of thrombosis and ischemic stroke.

Disclosure of Interest: None declared.

AS069

Tyrosine phosphorylation of the itim-containing receptor G6b-B is essential for platelet homeostasis

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Background: The immunoreceptor tyrosine-based inhibition motif (ITIM)-containing receptor G6b-B has been implicated as a critical regulator of platelet homeostasis. Tyrosine phosphorylation of the ITIM and immunoreceptor tyrosine-based switch motif (ITSM) in the cytoplasmic tail of G6b-B provides a docking site for the tyrosine phosphatases Shp1 and Shp2, which are thought to mediate downstream effects.

Aims: The aim of this study was to determine whether uncoupling of G6b-B from Shp1 and Shp2 abrogates G6b-B function *in vivo*.

Methods: We generated a mouse model in which tyrosine residues within the ITIM (Y212) and ITSM (Y238) of G6b-B were mutated to phenylalanines. Mice homozygous for the mutated *G6b* gene, referred to as *G6b-B diY/F*, were analysed for defects in platelet production and function.

Results: *G6b-B diY/F* mice exhibited a 75% reduction in platelet count and 38% increase in platelet volume, similar to that seen in *G6b* knockout (KO) mice. Extramedullary haematopoiesis and myelofibrosis was observed in both mouse models between 8 and 12 weeks of age. Platelet surface GPVI levels were similarly reduced by approximately 83% in both mouse models. In contrast, α IIb β 3 and GPIb α levels were increased by 106% and 36%, respectively, in *G6b-B diY/F* mice, which was not seen in *G6b* KO mice. Interestingly, G6b-B was up-regulated 175% in platelets from *G6b-B diY/F* mice, possibly as a compensatory mechanism for reduced downstream signaling. Platelets from these mice did not respond to the GPVI-specific agonist collagen-related peptide, and showed an attenuated response to PAR-4 peptide.

Conclusion: Loss of tyrosine phosphorylation of the ITIM and ITSM of G6b-B disrupts platelet homeostasis and recapitulates many of the features of *G6b* KO mice. The lack of a complete phenocopy of the two mouse models suggests residual signaling by G6b-B diY/F or the presence of biologically active splice variants of *G6b*.

This work was funded by the British Heart Foundation and the Medical Research Council.

Disclosure of Interest: None declared.

AS070

RASA3 is a critical inhibitor of platelet activation and the missing link in the P2Y12/RAP1 signaling pathwayStefanini L¹, Paul DS², Robledo RF³, Getz TM², Casari C², Piatt R², Boulaftali Y², Peters LL³ and Bergmeier W²¹Institute for Cardiovascular and Metabolic Research, University of Reading, Reading, UK; ²Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill; ³The Jackson Laboratory, Bar Harbor, USA**Background:** The small-GTPase RAP1 is a crucial regulator of platelet integrin activation and thrombus formation. RAP1 activity in platelets is controlled by the guanine nucleotide exchange factor CalDAG-GEFI and an unknown regulator operating downstream of the ADP receptor, P2Y12, a target of antithrombotic therapy.**Aims:** To evaluate the role of the GTPase-activating protein RASA3 in RAP1-dependent platelet activation and hemostasis.**Methods:** Germline or megakaryocyte-specific deletion of *Rasa3* in mice led to embryonic/perinatal lethality. Thus, platelet functional responses and the *in vivo* hemostatic response were examined in mice with reduced expression of RASA3 due to a non-tolerated point mutation (H794L, *Rasa3*^{h794l}).**Results:** Reduced expression of RASA3 led to severe thrombocytopenia due to systemic activation and premature clearance of platelets from circulation. The increased platelet turnover and the resulting thrombocytopenia were reversed by concomitant deletion of the RAP1 activator CalDAG-GEFI. Upon stimulation, *Rasa3* mutant platelets were hyperresponsive to agonists both *in vitro* and *in vivo*. Impaired RASA3 expression led to a state of genetically encoded P2Y12 signaling, where sustained activation of RAP1 and α IIb β 3 integrin in stimulated platelets did not require ADP feedback and was insensitive to P2Y12 or PI3-kinase inhibitors. Consistently, *Rasa3* mutant platelets formed very stable hemostatic plugs, even in the presence of the P2Y12 inhibitor, clopidogrel bisulfate.**Conclusion:** Our studies identify RASA3 as a critical inhibitor of platelet activation, and they suggest that RASA3 inactivation is the missing molecular link between P2Y12 engagement and sustained RAP1 activation in platelets. Our findings may lead to improved diagnosis and treatment of platelet-related disorders.**Disclosure of Interest:** None declared.

Mechanisms of ITP

AS071

T cells are critical players in the pathogenesis of immune thrombocytopenia (ITP)

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Immune thrombocytopenia (ITP) is a bleeding disorder in which autoantibodies and/or cytotoxic T cells are directed against an individual's own platelets and/or megakaryocytes which leads to enhanced peripheral platelet destruction and/or reduced bone marrow production, respectively. The cause of this disease is unknown and most of the original research in ITP concentrated on characterization of anti-platelet autoantibodies. In the last 25 years, however, an explosive body of literature has arisen to shed new light on the complex immunopathogenesis of ITP. It is associated with three interrelated areas of autoimmunity pertaining to environmental e.g. infectious influences, antigen presenting cell (APC) function and particularly, T cell abnormalities. It appears that the processes of apoptosis and deficiencies of CD4⁺ T regulatory cells are central events that lead to tolerance breakdown and induction of platelet autoimmunity. This lecture will update our understanding of the T cell-mediated immunology of ITP

and will highlight recent literature which suggests that pro-inflammatory forces lead to an environment that significantly dysregulates autoreactive T cells leading to anti-platelet autoimmunity.

Disclosure of Interest: None declared.

AS072

Altered plasma microRNA levels in patients with immune thrombocytopenic purpuraHe Y¹, Zuo B², Zhao Y³ and Wu Q⁴¹Jiangsu Institute of Hematology, the First Affiliated Hospital, Soochow University; ²Cyrus Tang Hematology Center, Soochow University; ³Jiangsu Institute of Hematology, the First Affiliated Hospital, Soochow University; ⁴Jiangsu Institute of Hematology, Cyrus Tang Hematology Center, the First Affiliated Hospital, Soochow University, Suzhou, China**Background:** Immune thrombocytopenic purpura (ITP) is a common bleeding disorder but the underlying disease mechanism remains poorly understood. MicroRNAs (miRNA) are important molecules in regulating gene expression. Altered miRNA expression has been implicated in a variety of human immune diseases.**Aims:** The goal of this study is to test the hypothesis that plasma miRNA levels may be altered in patients with ITP.**Methods:** Plasma samples from 10 adult ITP patients and 6 healthy controls were used as a discovery set for miRNA profiling. RNAs were extracted from plasma by miRNeasy mini kit (Qiagen) and analyzed by Agilent miRNA microarray. miRNAs with altered expression levels in samples from ITP patients compared with that in normal controls were selected and oligonucleotide primers specific for these miRNAs were made. Quantitative real-time PCR was performed to examine the level of the selected miRNAs in an independent validation sample set from 56 ITP patients and 52 healthy controls.**Results:** In the discovery set of plasma samples, a total of 53 miRNAs were found to have higher or lower expression levels (> 1.5 fold, $P < 0.05$) in ITP patients compared with that in normal controls. In the validation study, 3 miRNAs (miR-320c, miR-3667 and miR-4499) and 4 miRNAs (miR-223-3p, miR-652-5p, miR-142-3p and Let-7b) were confirmed to be up-regulated and down-regulated, respectively, in ITP patients. Among them, plasma levels of miR-320c were found to be negatively correlated with ($r = -0.560$, $P < 0.001$) blood platelet counts in ITP patients, whereas plasma levels of miR-223-3p were positively correlated with ($r = 0.753$, $P < 0.001$) blood platelet counts in ITP patients. Bioinformatic analysis suggested that miR-320c and miR-223-3p may target genes in the PI3K-Akt and Toll-like receptor signaling pathway, respectively.**Conclusion:** Our results indicate that plasma levels of miR-320c and miR-223-3p were altered in ITP patients, suggesting a potential mechanism of deregulated miRNA expression in the pathogenesis of ITP.**Disclosure of Interest:** None declared.

AS073

CD40L and its DNA methylation in primary immune thrombocytopenia

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Background: The CD40-CD40L co-stimulatory pathway plays a pivotal role in inflammation. CD40L expression is higher in immune thrombocytopenia (ITP) patients. However, the mechanism underlying

ing this overexpression remains unknown. Recent evidence indicated that DNA demethylation reactivates the silent X chromosome, resulting in CD40L overexpression and further contribute to autoimmunity.

Aims: Explore the expression and methylation status of CD40L in CD4⁺ T cells from ITP patients and analysis the effect of DNA demethylation on CD40L expression.

Methods: Fifty-six active ITP patients, 12 ITP patients in remission and 30 healthy controls were enrolled in this study. The plasma sCD40L level, CD40L protein and mRNA expression of the CD4⁺ T cells were detected by ELISA, Flow cytometric and real time PCR respectively. In addition, SEQUENOM method was performed to determine the methylation status of CpG sites in the CD40L promoter region from CD4⁺ T cells.

Results: (1) Active ITP patients had significantly elevated plasma sCD40L levels than those of remission patients and controls (both $P < 0.05$), but there is no difference between the female/male ITP patients and gender-matched controls. (2) The CD4⁺ CD40L⁺ T cell's percentage and the CD40L mRNA expression in CD4⁺ T cells were significantly elevated in female ITP patients compared with female controls ($P < 0.05$), and they were also higher in active ITP patients than those of patients in remission and controls (both $P < 0.05$). (3) The average methylation degree of each CG pair and methylation status of -71, -38, +78 and +110 CpG sites were higher in female controls than in male controls, but there is no differences revealed between female patients and female controls.

Conclusion: Abnormal expressed CD40L is involved in development of ITP, and over-expressed CD40L may be one of the reasonable explanations for female susceptibility to ITP. In addition, CD40L overexpression in CD4⁺ T cells from female patients with ITP is not associated with dysmethylation of CD40L promoter.

Disclosure of Interest: None declared.

AS074

Cyclosporine enhances immunosuppressive functions of myeloid-derived suppressor cells in immune thrombocytopenia

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Background: Immune Thrombocytopenia (ITP) remains the most common autoimmune bleeding disorder and cell-mediated platelet destruction is an important mechanism leading to thrombocytopenia. Myeloid-derived suppressor cells (MDSCs) is a heterogeneous group of immature cells and natural inhibitors of adaptive immunity.

Aims: We evaluated the MDSCs population in patients with primary ITP to demonstrate their role in the pathogenesis of ITP.

Methods: The phenotype as well as the expression of arginase-1 and iNOS of MDSCs in peripheral blood and splenic frozen sections from ITP patients or healthy controls were analyzed. Platelets co-cultured with MDSC-primed CD8⁺ T lymphocytes were tested for apoptosis using JC-1 mitochondrial membrane potential assay kit.

Results: Our data showed that the number of CD33⁺CD11b⁺HLA-DR⁻ MDSCs was lower in ITP patients; in these MDSCs, lower arginase-1 levels and higher iNOS levels were found. Cyclosporine enhanced suppressive functions of MDSCs induced from human PBMCs and splenocytes in a dose-dependent manner, exerting prominent inhibition of CD4⁺ T cell proliferation. Moreover, the expression of IL-10 and TGF- β was up-regulated significantly in cyclosporine-modulated MDSCs compared with the unmodulated systems. After cyclosporine modulation, MDSC-induced autologous T regulatory cells Tr1 increased significantly. Lower apoptosis level was demonstrated in platelets co-cultured with MDSC-primed CTLs compared

with those co-cultured with unprimed CTLs. Furthermore, cyclosporine enhanced the control of MDSCs over CTL-mediated platelet lysis.

Conclusion: These findings indicated the immunosuppressive effect of MDSCs in modulating T cell responses and cell-mediated platelet destruction. In conclusion, impaired MDSCs may be involved in ITP. Cyclosporine augmented suppressive activities of human MDSCs *in vitro*. Thus, targeting MDSCs may pave the way for further therapeutic options for patients with ITP.

Disclosure of Interest: None declared.

AS075

Elevated Sema5A in patients with immune thrombocytopenia

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Background: Immune thrombocytopenia (ITP) is an immune-mediated platelet disorder. Cytokine-mediated immunity and impaired cellular immunity play an important role in the pathogenesis of ITP. A recent study showed that Soluble Sema5A strongly increased T cell and NK cell proliferation and induced the secretion of proinflammatory Th1/Th17 cytokines. The role of sema5A in the pathogenesis of immune thrombocytopenia (ITP) remains unknown.

Aims: The aim of this study is to investigate the possible pathogenic role of Sema5A and its mechanisms in ITP.

Methods: Plasma Sema5A, interferon (IFN)- γ , IL-4, IL-17A were measured in patients with active ITP, remission and healthy controls by enzyme-linked immunosorbent assay. Using real-time quantitative polymerase chain reaction, the mRNA expression of sema5a, its receptor plexinb3, plexinA1 were studied in all subjects. Plasma Sema5a as well as sema5a, plexinB3, plexinA1 mRNA expression was determined in 9 ITP patients receiving Immune-Modulatory treatment.

Results: Significantly higher levels of plasma sema5a and plasma IFN- γ , lower level of IL-4 were detected in active ITP patients compared with healthy controls or patients in remission. A positive correlation between sema5a and plasma IFN- γ was found in ITP patients with active disease. In addition, significantly lower plexinB3 mRNA expression was observed in active ITP patients compared with healthy controls or patients in remission. A negative correlation was found between the level of plasma sema5a and plexinb3 mRNA expression levels in active ITP patients. The level of plasma sema5a significantly decreased in 9 patients after immune regulatory treatment, concurrently plexinB3 mRNA significantly increased.

Conclusion: This study is the first in which sema5A is shown to play a role in the pathogenesis of ITP. These findings along with the observation of sema5A being increased in ITP patients suggest this regulatory molecule as a therapeutic agent for ITP to be assessed in the future.

Disclosure of Interest: None declared.

Tools for evaluation of bleeding

AS076

The clinical utility of bleeding scores

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The accurate evaluation of hemorrhagic symptoms is a key component in the diagnosis of mild bleeding disorders. Bleeding histories present well-recognized challenges for both patients and physicians however,

as significant symptoms may be overlooked because they are considered normal and minimal or trivial symptoms may be given undue consideration. In response to these challenges, bleeding assessment tools (BATs) have been developed and validated in an attempt to: (1) standardize bleeding histories using a bleeding score, (2) improve diagnostic accuracy, (3) describe symptom severity, (4) predict the risk of bleeding in a individual patient and (5) inform treatment. This work has been pioneered by a group of Italian researchers, and the resultant 'Vicenza Bleeding Questionnaire' stands as the original BAT. In this presentation, the modifications to the Vicenza Bleeding Questionnaire that have taken place over the years will be discussed, as well as the validation studies that have been published. Other BATs that have been developed and published will be reviewed, as will the special situations of assessing pediatric bleeding as well as menorrhagia. To date, BATs have been studied in a variety of clinical settings including primary and tertiary care clinics and in both pediatric and adult populations. Heterogeneity exists between studies and their results, although there are commonalities, most notably the ability of BATs to distinguish normal and abnormal bleeding. Bleeding score 'saturation' in individuals with more severe bleeding remains a challenge, as does the inflexibility of the current scoring systems to changes in bleeding patterns over time. Future directions for bleeding scores include the development of self-administered BATs to reduce burden on experts during clinic and provide potential assistance to diagnosing unrecognised individuals suffering from abnormal bleeding.

Disclosure of Interest: P. James Grant/Research Support from: CSL Behring, Octapharma, Bayer.

AS077

Accuracy of three clinical prediction tools for major bleeding in patients on extended oral anticoagulant for venous thromboembolism

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Background: Tools exist to predict the risk of major bleeding in patients on oral anticoagulation therapy (OAT) for venous thromboembolism (VTE) but these have focused on the higher risk, first 3 months of therapy (risk of major bleeding is 2.4% in the first 3 months, vs. 2% per year thereafter), and have not been developed or evaluated for bleeding risk after the first 3 months of therapy (extended OAT).

Aims: We sought to evaluate the rates of major bleeding predicted by three previously published tools (outpatient bleeding risk index [OBRI], RIETE, and HAS-BLED) in patients with VTE during extended OAT.

Methods: The Bleeding Risk study is a multicentre, multinational prospective cohort study of patients on extended OAT for unprovoked VTE, or provoked VTE with prior VTE. Patients were enrolled after at least 3 months of OAT. All major bleeding events during long term OAT were captured and adjudicated. Scores were assigned using the 3 tools to estimate the risk of major bleeding.

Results: To date, 2525 patients enrolled at 12 sites have contributed 7549 years of observation. 91 patients (3.6%) experienced at least one

episode of major bleeding. The proportion of patients classified as low probability of bleeding by the OBRI, RIETE and HAS-BLED scores were 51.0%, 49.9%, and 63.3%, with major bleeding rates of 0.75%, 0.53%, and 0.74% per year, respectively. The percentage of patients who were classified as intermediate probability of bleeding were 47.8%, 49.7%, and 33.0% with major bleeding rates of 1.7%, 1.8%, and 1.8% per year respectively. Only 1.2%, 0.4%, and 3.8% of patients were classified as high risk of bleeding according to the three tools and major bleeding rates were 2.8%, 7.2%, and 3.6%, respectively.

Conclusion: The three tools discriminate bleeding risk and accurately predict that most VTE patients on extended OAT have a low risk of major bleeding. However, they identify very few patients with a high risk of major bleeding on extended OAT for VTE.

Disclosure of Interest: None declared.

AS078

Utility of bleeding scores and a standardized laboratory evaluation in identifying defects in primary hemostasis in children referred to a tertiary-care pediatric centre

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Background: Mucocutaneous bleeding occurs frequently during childhood but diagnosing defects in 1° hemostasis remains challenging.

Aims: To identify the spectrum of defects in 1° hemostasis in children referred to a tertiary-care pediatric clinic because of mucocutaneous bleeding symptoms and/or a family history of platelet dysfunction or von Willebrand disease (VWD).

Methods: Prospective, standardized evaluation according to a diagnostic algorithm (Israel et al., *Pediatr Blood Cancer* 2011; 56: 975) that includes determination of bleeding scores and repeat determinations of platelet count, VW factor panel, and platelet aggregation and ATP secretion testing. The study was approved by the local ethics committee and written informed consent was obtained.

Results: We investigated 116 children, 59% female, with a median age of 11.0 years (range: 0.6–17.8 years). The median PBQ (Pediatric Bleeding Questionnaire) score was 4 (range: 0–14) and the median ISTH-BAT (Bleeding Assessment Tool) score was 3 (range: 0–13). The PBQ score was normal (< 2) in 12 children; 36 had a normal ISTH-BAT score (< 3).

Laboratory results were normal in 78 children. Platelet dysfunction was diagnosed in 11 children, possible platelet dysfunction in 5, and 2 children had thrombocytopenia. VWD was diagnosed in 15 children, 1 of whom also had platelet dysfunction, and another, heterozygous factor (F) XI deficiency. In addition, 5 children were diagnosed with a defect in 2° hemostasis: heterozygous FVII (*n* = 2) or FXI (*n* = 1) deficiency; hemophilia A carrier (*n* = 2).

A 1° hemostasis defect was found in 3/12 and 7/36 of the children with normal PBQ and ISTH-BAT scores, respectively; 1 child with FVII deficiency had normal PBQ and ISTH-BAT scores and 1 hemophilia A carrier had a normal ISTH-BAT score only.

Conclusion: A standardized algorithmic approach allowed diagnosis of a 1° hemostasis defect in 28% of children referred to a tertiary-care pediatric clinic, with 91% of these children having a positive PBQ score and 79%, a positive ISTH-BAT score.

Disclosure of Interest: None declared.

AS079

Assessing bleeding phenotype in children with moderate or severe von Willebrand disease using the ISTH-BAT and the relevance of pediatric-specific bleeding symptoms

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Background: The bleeding phenotype of von Willebrand disease (VWD) in children may differ from that in adults, and data in children is limited. The value of the ISTH bleeding assessment tool (ISTH-BAT) has only been demonstrated in children with mild VWD. This is the first study to evaluate the ISTH-BAT in a large cohort of children with moderate and severe VWD.

Aims: To assess the bleeding phenotype and to evaluate the ISTH-BAT in a large cohort of children with moderate and severe VWD.

Methods: Of 140 children aged 0–16 at inclusion in the nation-wide cross-sectional 'Willebrand in the Netherlands' (WiN) study with VWF antigen and/or activity levels ≤ 30 IU dL⁻¹, 133 with type 1 ($n = 60$), 2 ($n = 44$) and 3 ($n = 9$) VWD were available for this study. Bleeding severity was determined using the ISTH-BAT with extra pediatric-specific bleeding symptoms (bleeding after vaccination, heel prick test, fetal scalp electrode and excessive bruising after birth) administered by a trained physician (YS). Medical Ethics Committee approval and informed consent were obtained.

Results: All post-menarche girls suffered from menorrhagia (26/26). Other frequently occurring bleedings were cutaneous bleeding (81%), oropharyngeal bleeding (64%), prolonged bleeding from minor wounds (58%) and epistaxis (56%). Pediatric-specific bleeding symptoms had been experienced by 44% of the patients, occurring in 48% of type 1, 32% of type 2, and 78% of type 3 patients. ISTH-BAT score was higher in type 3 VWD than in type 2 or 1 (17 vs. 10.5 vs. 6.5, $P < 0.001$) and higher in children with severe VWD (VWF levels < 10 IU dL⁻¹) vs. moderate VWD (VWF levels 10–30 IU dL⁻¹; 11 vs. 7, $P < 0.001$) and higher in index cases than in affected family members (12 vs. 6.5, $P < 0.001$).

Conclusion: The ISTH-BAT score is associated with type and severity of VWD and can be used in the evaluation of children with suspected VWD. In our cohort of children with moderate and severe VWD, almost half suffered from pediatric-specific bleeding symptoms, underlining the relevance of these data.

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Cancer and Thrombosis Clinical

AS080

Controversies in cancer-associated thrombosis

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Thromboembolism is the second most common cause of death and dramatically worsens cancer prognosis. Parenteral anticoagulation using low-molecular-weight-heparin is the current preferred management option for patients with acute cancer-associated thrombosis. Recently, the introduction of the direct oral anticoagulants has expanded the different therapeutic options for the management of venous thromboembolism but their efficacy and safety in cancer patients remains uncertain. The length of anticoagulation for patient with cancer-associated is also controversial and the acceptability of long-term treatment, especially with parenteral agents, needs put in the contexts of living with cancer and quality of life. Furthermore, the management of cancer-associated thrombosis is complex and clinicians are frequently confronted to complications including recurrent venous thromboembolism despite anticoagulation, thrombocytopenia, incidental thrombosis, etc. Unfortunately, there is a paucity data on how to manage these important clinical challenges and clinicians often have to rely on expert opinions. The aim of the presentation is to review these controversies and help clinicians to tailor their management of cancer-associated thrombosis based on patients' characteristics, preferences and complications.

Disclosure of Interest: M. Carrier Grant/Research Support from: LEO Pharma, BMS, Speaker Bureau of: Sanofi, Pfizer, Bayer, BI.

AS081

Tissue factor (TF) as predictor of recurrent venous thromboembolism (VTE): risk factor and biomarker analysis from the catch trial of treatment of cancer-associated VTE with tinzaparin or warfarin

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Background: Predictors of recurrent VTE (rVTE), unlike primary VTE, in patients with malignancy are incompletely understood. The recently completed CATCH trial collected baseline blood samples to evaluate selected biomarkers as predictors of rVTE.

Aims: We evaluated the association of TF, FVIII, D-dimer, sP-selectin and C-reactive protein (CRP) measured at time of initial VTE diagnosis with subsequent VTE.

Methods: The CATCH study was a randomized, multicenter trial (NCT01130025) of 900 patients comparing tinzaparin 175 IU kg⁻¹ once daily or dose-adjusted warfarin for 6 months in patients with cancer and acute, symptomatic VTE. Assays included TF ELISA and sP-selectin (R&D Systems), D-dimer (Roche), FVIII and CRP (Siemens). A Fisher's exact test was used to screen for association with rVTE; competing risk regression analysis was then conducted accounting for multiple variables.

Results: Study population comprised up to 805 patients with available biospecimens; 72 developed rVTE. Mean and median TF levels were 72.5 and 50.3 pg mL⁻¹ (range, 15.6–4798 pg mL⁻¹). VTE recurred most in highest quartile of TF (> 64.6 pg mL⁻¹; $n = 38/203$, 19% vs. $n = 34/599$, 6%; relative risk [RR] 3.3; 95% confidence interval [CI] 2.1–5.1; $P < 0.0001$). Elevated CRP (> 75 mg L⁻¹) was also associated with rVTE ($n = 482$; RR 2.3; 95% CI 1.3–4.2; $P = 0.007$) and a non-significant trend was observed with elevated D-dimer (> 5800 µg L⁻¹ FEU; $n = 780$; RR 1.6; 95% CI 1.0–2.5; $P = 0.08$). sP-selectin and FVIII were not significantly associated. In competing risk regression analysis, adjusting for treatment assignment, region, history of VTE and metastatic disease, TF continued to be strongly associated with risk of rVTE (adjusted hazard ratio 3.4; 95% CI 2.1–5.5; $P < 0.001$).

Conclusion: This is the first report to suggest that TF is a clinically significant biomarker predictive for rVTE in malignancy. A biomarker-based strategy could help identify high-risk patients benefiting from longer or more intense anticoagulation strategies.

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AS082

Extensive screening based on 18F-FDG PET/CT for occult malignant disease in unprovoked venous thromboembolism: a prospective randomized clinical trial

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Background: The association between venous thromboembolism (VTE) and cancer has been widely described. Approximately 10% of patients with unprovoked VTE will be diagnosed with cancer after 1 year. However, clear diagnostic work-up guidelines are not yet available. FDG PET/CT could serve as a comprehensive screening strategy for occult malignancy in patients with unprovoked VTE.

Aims: To compare an extensive screening strategy based on FDG PET/CT with a basic screening for detection of malignancies in patients with unprovoked VTE.

Methods: Patients with unprovoked VTE were included in a multicenter clinical trial, and randomized to either a basic screening strategy (physical examination, usual laboratory tests and basic X-Rays) or an extensive screening that consisted of the basic strategy plus a FDG PET/CT. The primary outcome was the rate of cancer diagnosed in each arm at inclusion. Patients were followed up during 2 years.

Results: A total of 399 patients were included, but 5 patients later withdrew their consent. Of the 394 patients, 197 were allocated in each arm. In intention-to-treat analysis, cancer was diagnosed in 11/197 patients (5.6%) in FDG PET/CT strategy group vs. 4/197 patients (2%) in standard strategy group, absolute risk difference 3.6% (95% CI -0.4% to +7.9%; $P = 0.07$). 26 patients did not received allocated intervention, 25 in FDG PET/CT group. In per protocol analysis, cancer was diagnosed in 9/172 patients in the FDG PET/CT group vs. 3/196 in standard strategy group ($P = 0.046$). The risk of subsequent cancer diagnosis over the 24 months follow-up period was 1/186 (0.5%) in the FDG PET/CT group, vs. 9/193 (4.7%) in the basic screening group, absolute risk difference 4.1% (95% CI 0.8–8.4; $P = 0.01$).

Conclusion: The use of FDG PET/CT was associated with a non-significant increase in the rate of cancer diagnosis at the time of unprovoked VTE. The risk of subsequent cancer diagnosis was significantly lower in patients after a negative screening strategy that included a FDG PET/CT (NCT00964275).

Disclosure of Interest: None declared.

AS083

Impact of initial cancer stage on the risk of venous thromboembolism: the Scandinavian Thrombosis and Cancer (STAC) Study

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Background: Cancer patients, who develop venous thromboembolism (VTE) have reduced survival compared to those who do not develop VTE. Recent studies indicate that initial cancer stage was strongly associated with development of VTE. The impact of cancer stage on risk of VTE has not yet been addressed in large population based cohort studies.

Aims: To investigate the association between VTE and cancer stages in specific types of cancers in a prospective cohort study, the Scandinavian Thrombosis and Cancer (STAC) study.

Methods: The STAC Study includes 144,952 participants from three prospective population based cohort studies, i.e. the Tromsø Study, the HUNT2 Study, and the Diet, Cancer and Health Study. The participants were enrolled during 1993–1997 and the mean follow-up time was 10 years. First time objectively confirmed VTE events during follow-up have been identified in prior studies. For this study we collected data on cancer diseases and stages of the cancers from the national cancer registries. We used Cox regression models to estimate the relative risk of VTE in cancer patients at different stages. Age was used as time axis to prevent confounding by age.

Results: Excluding participants with a VTE event more than 1 year before a cancer diagnosis left 19,955 participants with a cancer diagnosis for follow-up. Of these 1887 were lung cancer, 2807 were colo-rectal cancer, 3170 were breast cancer, and 3166 were prostate cancer patients. Regional spread of cancer was associated with a 2–3 times higher risk of VTE in lung and colo-rectal cancer but was not associated with a higher risk in breast and prostate cancer. Distant metastasis was associated with 2–7 times higher risk compared with localized cancer.

Conclusion: Our findings show the general tendency that advanced cancer stage is associated with higher risk of VTE but the risk estimates for regional and distant spread differed substantially for different types of cancer.

Disclosure of Interest: None declared.

AS084

Risk of recurrent venous thromboembolism in patients with active cancer

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Background: Population studies on the risk of recurrent venous thromboembolism (VTE) in patients with of a first cancer-associated VTE are limited. Recurrence rates are increased but there are no published data describing the incidence rates (IR) for recurrent VTE over time since the first VTE.

Aims: N/A.

Methods: A population based observational cohort study was undertaken to estimate the incidence of recurrent VTE in patients with a first active cancer-related VTE. The source population consisted of all patients in the UK Clinical Practice Research Datalink, with additional linked information on hospitalisations and cause of death. All first and recurrent VTE events between 2001 and 2011 were identified using validated algorithms. VTE events were defined as cancer-related if a clinical diagnosis or therapy for cancer was recorded in the 90 days before or after the VTE. Patients were followed up for their risk of recurrent VTE.

Results: There were 6592 active cancer-associated first VTE, 3055 (46.3%) deep vein thrombosis (DVT) and 3537 (53.7%) pulmonary embolism (PE). In a total of 6175 person years (PY) of observation after the first VTE 591 recurrent VTEs were observed (48.4% DVT and 51.6% PE). The IR of VTE recurrence was 9.6 per 100 PY, 8.8 per 100 PY after initial DVT and 10.5 per 100 PY after PE. The IR peaked at 22.6 per 100 PY in the first 6 months, fell to 8.6 between 6 and 12 months and further decreased over the next 5 years.

Conclusion: VTE in patients with active cancer is common and associated with high recurrence rates. Efforts are needed to prevent VTE and to reduce recurrences, especially in the first year following VTE diagnosis.

Disclosure of Interest: A. Cohen Consultant for: Bayer, Boehringer-Ingelheim, BMS, Daiichi, GSK, Johnson and Johnson, Mitsubishi Pharma, Pfizer, Portola, Sanofi, Schering Plough, Takeda, XO1, UK Government Health Select Committee, the Department of Health, the NHS, Lifeblood and the Coalition to Prevent Venous Thromboembolism. S. Rietbrock Grant/Research Support from: IfESI has received grants for investigator-initiated studies from Bayer Pharma AG, BMS-Pfizer and CSL Behring., A. Katholing Grant/Research Support from: IfESI has received grants for investigator-initiated studies from Bayer Pharma AG, BMS-Pfizer and CSL Behring., C. Martinez Grant/Research Support from: IfESI has received grants for investigator-initiated studies from Bayer Pharma AG, BMS-Pfizer and CSL Behring., Consultant for: Bayer Pharma AG and Boehringer Ingelheim.

Vascular Progenitor Cells and Vascular Disease

AS085

Endothelial progenitors cells: from pathophysiology to emergent therapy of vascular disorders

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Dysfunction of endothelium is a primary determinant of cardiovascular diseases, namely atherosclerosis and its ischemic complications. However, poor accessibility to patient specific endothelium is an

important limitation for studying the endothelial alterations and designing potential targeted therapies. In the last 10 years, endothelial precursors have been identified among circulating blood cells and shown to be key effectors able to replace damaged endothelial cells and to contribute to *de novo* vessel growth. Due to their clonogenic proliferative potential, these precursors can be used to generate *in vitro* a typical progeny of phenotypically and functionally competent endothelial cells. We previously used the ECFC model to investigate the molecular mechanisms that may link prematurity or low birth weight (LBW) to early vascular dysfunction and enhanced cardiovascular risk. We reported a significant reduction of the vasculogenic properties of ECFC isolated from cord blood of preterm and LBW neonates, resulting from a thrombospondin 1-dependent angiostatic phenotype. In addition a SIRT1 deficiency drives an accelerated senescence in LBW-ECFC that acts as a critical determinant in impairment of endothelial repair capacity. More recent data evidenced that SIRT1 deficiency in LBW-ECFC activates p38 MAPK-dependent signaling pathways that result in increased release of extracellular vesicles. The senescence-induced microparticles take part of a proinflammatory secretome and are able to transfer senescence phenotype into naive endothelial cells. Interestingly, resveratrol treatment was shown to reverse ECFC defects laying new grounds for a pharmacological control of early programmed vascular risk. These data show that ECFC now provide relevant resources for probing endothelial alterations but also to develop cell based vascular therapy, in the context of specific patients' genetic and environmental backgrounds.

Disclosure of Interest: None declared.

AS086

CD34⁺ hematopoietic stem cell count is predictive for vascular event occurrence in sickle cell disease children

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Background: Identifying sickle cell disease (SCD) children at neurovascular risk is currently well assessed by transcranial doppler screening. However, peripheral vascular risk remains difficult to evaluate and there is a need for relevant predictive biomarkers.

Aims: In SCD children explored at steady-state, our aim was to highlight a predictive biomarker for the occurrence of further vascular events.

Methods: SCD children aged 3–18 years old were consecutively included at steady state. Controls matched for age and ethnicity were recruited. Hematology, coagulation, hemolysis, endothelial and vascular parameters were recorded at inclusion. Circulating endothelial cells (CEC) count used immunomagnetic separation. CD34⁺ hematopoietic stem cells were enumerated (cells mL⁻¹) by flow cytometry. Neurovascular and peripheral events were prospectively recorded during the follow-up period, which continued 18 months after the last inclu-

sion. The local institutional ethical review committee approved the study protocol.

Results: We enrolled 108 SCD patients at steady state (sex ratio 0.61, mean age 9.9 years). Compared to controls, SCD patients at steady-state displayed elevated markers of hemolysis and platelet activation, significant higher leukocyte, CD34⁺ and microparticle counts, and a pro-coagulant profile. Patients did not differ from controls in terms of CEC or nucleosome level. Thirty-six patients had at least one neurovascular ($n = 12$) or peripheral vascular event ($n = 25$) during a mean follow-up of 27 months. In a multivariate model, higher CD34⁺ cell count was the best predictor for the occurrence of a vascular complication (OR 1.2 for 1000 cell mL⁻¹ increase, 95% CI [1.049–1.4], $P = 0.013$, sensitivity 53%, specificity 84% for a threshold of 8675 cells mL⁻¹). No hemolysis or coagulation parameter was predictive of vascular event occurrence after multiplicity correction.

Conclusion: CD34⁺ cell count is a promising biomarker of further vascular event occurrence in SCD children at steady-state.

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AS087

Treprostinil indirectly regulates endothelial colony forming cell angiogenic properties by increasing VEGF-A produced by mesenchymal stem cells

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Background: Pulmonary vasodilators and prostacyclin therapy in particular, have markedly improved the outcome of patients with pulmonary hypertension (PH). Endothelial dysfunction is a key feature of PH, and we previously reported that treprostinil therapy increases number and proliferative potential of endothelial colony forming cells (ECFC) isolated from PH patients' blood.

Aims: In the present study, objectives were to determine how treprostinil contributes to the proangiogenic functions of ECFC.

Methods: We examined the effect of treprostinil on ECFC obtained from cord blood in terms of colony numbers, proliferative and clonogenic properties *in vitro*, as well as *in vivo* vasculogenic properties.

Results: Surprisingly, treprostinil inhibited viability of cultured ECFC but did not modify their clonogenic properties nor their endothelial differentiation potential from cord blood stem cells. Treprostinil treatment significantly increased the vessel-forming ability of ECFC combined with mesenchymal stem cells (MSC) in Matrigel implanted in nude mice. *In vitro*, ECFC proliferation was stimulated by conditioned media from treprostinil-pretreated MSC, and this effect was inhibited either by the use of VEGF-A blocking antibodies or siRNA VEGF-A in MSC. Silencing VEGF-A gene in MSC also blocked the pro-angiogenic effect of treprostinil *in vivo*. The clinical relevance of these data was confirmed by the high level of VEGF-A detected in plasma from patients with pediatric pulmonary hypertension who had been treated with treprostinil.

Conclusion: In conclusion, increased VEGF-A produced by MSC can account for the increased vessel formation observed during treprostinil treatment. Moreover, our results suggest that VEGF-A level in patients could be a surrogate biomarker of treprostinil efficacy.

Disclosure of Interest: None declared.

AS088

Induced pluripotent stem cell (iPSC) based strategy to correct the bleeding phenotype in hemophilia A

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Background: Hemophilia A (HA) is a bleeding disorder caused by factor (F) VIII gene mutations. Somatic cells can be reprogrammed to generate autologous, disease-free iPSCs, and differentiated into cell types relevant for gene and cell therapy.

Aims: HA treatment strategy approach generating patient-specific iPSCs from peripheral blood cells differentiating them into endothelial cells (ECs) secreting FVIII.

Methods: Mononuclear and CD34⁺ cells were isolated from healthy and HA donors and reprogrammed with a Cre-LoxLentivector (LV-OCT4-SOX2-KLF4). iPSCs were characterized by Alkaline Phosphatase, immunofluorescence (IF), telomeres length, RT-PCR for stem cell markers and methylation of Nanog-promoter. On embryoid bodies, germ layers markers expression and differentiation potential were assessed. iPSCs were differentiated in ECs and markers expression was evaluated by FACS and RT-PCR. ECs were transduced with LV carrying GFP under the control of EC-specific promoters and transplanted in monocrotaline-conditioned NSG mice. ECsliver engraftment was analyzed by GFP-staining. HA-ECs cells were corrected using the same LV with GFP replaced by FVIII and injected *in vivo*.

Results: Reprogrammed cells gave rise to *bona fide* iPSCs colonies. Telomeres length increased in iPSCs and decreased in iPSCs-derived-ECs. No abnormalities were detected at karyotype analysis. ECs expressed EC-markers and formed tubules when cultured in matrigel. RT-PCR and IF on HA-ECs showed hBDD-FVIII expression, confirming genetic correction by LV. Transplanted GFP+ EC were detected by IF on liver sections up to 6 weeks post transplantation. Therapeutic correction was reached in a short term experiment (up to 1w) after ECs injection in the peritoneum of NSG-HA mice.

Conclusion: These data will be instrumental to assess engraftment, proliferation and FVIII expression from differentiated, gene corrected and reprogrammed factor-free iPSCs to confirm the suitability of this approach for HA gene-cell-therapy.

Disclosure of Interest: None declared.

Vessel wall in health and disease annexins

AS089

Annexin A2 in health and disease

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Annexin A2 (A2) is a multicompartamental, multifunctional protein that orchestrates a growing spectrum of physiologic and pathophysiologic processes related to its calcium-sensing, phospholipid-binding function. At the endothelial cell surface, annexin A2 and S100A10 form the heterotetrameric (A2-p11)₂ complex, which binds both plasminogen and tissue plasminogen activator, thereby accelerating activation of the fibrinolytic protease, plasmin. Annexin A2-deficient mice display microvascular fibrin accumulation, have a prothrombotic response to vascular injury, and show defective postnatal angiogenesis.

In humans with antiphospholipid syndrome, high titer anti-A2 antibodies are associated with clinical thrombosis, and anti-A2 antibodies have also been found in a cohort of subjects with cerebral venous thrombosis. Over-expression of annexin A2 occurs in acute promyelocytic leukemia (APL) blasts, and appears to contribute to the bleeding diathesis of APL. Interestingly, expression of annexin A2 is upregulated under hypoxic conditions, and *Anxa2*^{-/-} mice are resistant to hypoxia-induced retinal neovascularization, a system that mimics some aspects of diabetic retinopathy. In a model of aseptic wear debris-mediated arthritis, intracellular annexin A2 maintains late lysosomal membrane integrity, thereby modulating inflammasome assembly and activation, and enabling subsequent cytokine secretion. Similarly, in a model of inflammatory bowel disease, annexin A2 modulates the innate inflammatory response of macrophages by promoting lysosomal membrane integrity and preventing release of lysosomal hydrolases into the cytoplasm. Together, these findings suggest emerging, multifaceted roles for annexin A2 in hemostasis, angiogenesis, and the innate inflammatory response.

Disclosure of Interest: None declared.

AS090

The endothelial cell transcriptome is highly heterogeneous across different vascular beds

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Background: Endothelial cells (ECs) display remarkable structural and functional heterogeneity. Although a number of EC specific genes have been identified, data on the overall EC transcriptome is limited, mainly because of difficulties isolating ECs from complex tissues and the phenotypic drift associated with manipulation and expansion of ECs *in vitro/ex vivo*.

Aims: To characterize the EC transcriptome *in vivo* using an unbiased genome-wide approach.

Methods: We applied an *in vivo* system in which a conditional hemagglutinin-epitope tag is targeted into the mouse ribosomal protein *Rpl22* locus and activated specifically in ECs to immunoprecipitate ribosome-associated mRNA. Using this method, we previously demonstrated that *F8* mRNA expression is restricted to ECs (Everett et al. Blood 2014). We now extend this analysis to characterize EC mRNA isolated from brain, heart, kidney, liver and lung by high-throughput RNAsequencing (RNASeq). In addition, we analyzed *Rpl22* tagged and untagged mRNA isoforms to calculate the size of the EC fraction in each of these tissues.

Results: *Rpl22* isoform analysis demonstrated that ECs comprise variable fractions in tissues, ranging from 3% (liver) to 23% (lung). RNA-Seq analysis identified a set of pan-endothelial genes, including both established markers as well as genes previously not associated with ECs. Additionally, each tissue exhibited a set of vascular bed-specific EC transcripts, which was most prominent in brain. A high degree of correlation was observed between quantitation by qPCR and RNASeq ($r = 0.90$), and confirmed the presence of established pan-EC markers such as *Cdh5*, *Tek*, *Vcam1* and *Vwf*.

Conclusion: ECs among different vascular beds are highly heterogeneous in their expression programs. These differences likely identify a range of specific transcripts unique to each distinct tissue and lay the foundation for future studies exploring changes in the endothelium under physiologic and pathologic conditions.

Disclosure of Interest: None declared.

AS091

A class of biased PAR1 agonists that stimulate cytoprotective signaling in endothelial cells

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Background: Stimulation of endothelial PAR1 by activated protein C (APC) triggers a cytoprotective pathway that protects from inflammation-induced apoptosis and barrier dysfunction. APC was used to treat sepsis. However, its anticoagulant properties limited the concentrations that could be administered, diminishing its utility. We have discovered a group of small molecules termed parmodulins (PMs) that act at the cytosolic face of PAR1 and affect some downstream signaling pathways, but not others.

Aims: To determine whether PMs activate a cytoprotective signaling pathway in endothelium.

Methods: Effects of PMs on endothelial signaling were evaluated *in vitro* and *in vivo*.

Results: Incubation with PMs protected endothelial cells against TNF α -induced apoptosis. Knockdown of PAR1 confirmed that the protective effect of PMs was mediated by PAR1. Evaluation of downstream signaling showed that PMs induced activation of Rac1. Rac1 activation can block TNF α -induced stimulation of NF κ B. Consistent with such inhibition, PM exposure blocked TNF α -induced, NF κ B-mediated transcriptional activation as detected using a GFP reporter construct. Transcript profiling showed that PM2 exposure resulted in upregulation of 26 genes, including stanniocalcin-1 (SCT1), a protein invoked in endothelial cytoprotection. Exposure of HUVECs to PMs increased expression of SCT1 mRNA and protein. SCT1 knockdown reversed the protective effect of PM2 against TNF α -induced apoptosis. Infusion of PM2 into mice resulted in increased expression of SCT1 in endothelial cells as detected in aortic slices. PM2 infusion also decreased surgery-induced leukocyte rolling on mouse venules to 41% of control ($P < 0.03$). PM2 infusion did not affect bleeding times.

Conclusion: PMs are the first compounds identified to activate a cytoprotective program in endothelial cells through PAR1. These biased PAR1 endothelial cell agonists represent a new strategy in the treatment of inflammatory disorders and sepsis.

Disclosure of Interest: None declared.

AS092

Podoplanin and CLEC-2 drive cerebrovascular patterning and integrity during development

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Background: Mice with a constitutive or platelet-specific deletion of the C-type lectin-like receptor, CLEC-2, which is expressed at high levels on platelets, exhibit cerebral haemorrhaging by mid-gestation. The only known endogenous ligand for CLEC-2, podoplanin, is expressed throughout the developing neural tube at embryonic day (E)10.5, concurrent with neural tube vascularisation.

Aims: We sought to investigate the basis of this phenotype, hypothesizing that CLEC-2 activation by podoplanin is required to maintain the integrity of developing cerebral blood vessels.

Methods: *Podoplanin*^{fl/fl} mice were generated and crossed to PGK-Cre or Nestin-Cre mice, to mediate constitutive or neural-specific deletion,

respectively. The developing vasculature was visualised in 3-dimensions using planar illumination microscopy and the ultrastructure of vessels was investigated by electron microscopy. To dissect out a mechanism behind the defect, a series of transgenic mouse models were used.

Results: Loss of podoplanin or CLEC-2 resulted in the formation of tortuous, aberrantly patterned cerebral blood vessels at E10.5, which preceded the development of haemorrhages throughout brain by E12.5. At E11.5, a defect in pericyte recruitment to cerebral blood vessels was observed and electron microscopy revealed large dissociations between the endothelium and its surrounding cells. Cerebral haemorrhages were also observed at E12.5 in mice deficient in the platelet glycoprotein IIb, and mild haemorrhaging was seen at E12.5 in neurobeachin-like-2 (NBEAL-2)-deficient mice, which lack platelet α -granules.

Conclusion: This study reveals a novel role for podoplanin and CLEC-2 in maintaining the integrity of the developing cerebral vasculature. It is proposed that podoplanin on neuro-epithelial cells induces platelet activation through CLEC-2, mediating platelet adhesion and aggregation to plug the vessel wall, while secreted molecules may be involved in recruiting mural cells to the endothelium, together preventing lethal haemorrhage.

Disclosure of Interest: None declared.

Cancer and thrombosis Basic

AS093

Platelets in cancer progression and metastasis

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There is an increasing body of experimental and clinical evidence that platelets and hemostatic system in general directly influence tumor growth and, possibly, tumor metastasis. We show that distal tumors are able to communicate with bone marrow and promote release and recruitment of Bone Marrow Derived Cells (BMDCs) to promote tumor angiogenesis. Platelets facilitate communication between the tumor and the bone, and mediate the release of proangiogenic BMDCs into the circulation. Platelet α -granule secretion, but neither platelet aggregation nor dense granule secretion is responsible for the mobilization of BMDCs and subsequent augmentation of tumor angiogenesis. Using several tumor models, including prostate cancer and melanoma, we demonstrate that growing tumors promote premetastatic changes in the bone resulting in the changes of bone structural indices as documented by microCT. Platelet depletion almost completely blocked tumor-induced changes in bone based on bone volume and bone surface area measurements demonstrating that platelets are necessary for tumor-induced bone remodeling prior to metastasis. Tumors secrete a number of factors which can be sequestered by platelets and released upon platelet activation. Several tumor-derived factors are present exclusively in platelets but not in plasma of tumor-bearing animals. Platelets of tumor-bearing animals contain high amounts of factors controlling bone metabolism and, potentially, bone metastasis. TGF- β 1, known to promote skeletal metastasis of several cancers, is present at the highest concentration in platelets and the concentration of TGF- β 1 increased ~100 fold in the presence of a tumor. Neutralization of TGF β 1 in platelet releasates from tumor-bearing mice reduces osteoblast differentiation, thereby directly implicating platelet-stored TGF β 1 in tumor-induced bone remodeling. Thus, platelets mediate communications between growing tumor and its micro and macro environment.

Disclosure of Interest: None declared.

AS094

GPV is a central regulator of hemostasis, thrombosis and thrombo-inflammatory brain infarction in miceSchießl S¹, Stegner D¹, Hagedorn I¹, Kraft P², Thielmann I¹, Stoll G² and Nieswandt B¹¹Chair of Experimental Biomedicine – Vascular Medicine, University Hospital, Rudolf Virchow Center of Experimental Biomedicine, University Wuerzburg; ²Department of Neurology, University Hospital, Wuerzburg, Germany

Background: Glycoprotein (GP) V is part of the GPIb-V-IX complex which mediates the initial adhesion of platelets to the exposed extracellular matrix upon vessel wall injury via the GPIb α subunit. GPV can be cleaved by thrombin, but – despite the existence of two knockout strains – the function of GPV remains elusive.

Aims: We aimed to elucidate the role GPV and its cellular regulation in thrombosis and thrombo-inflammatory processes. Therefore, mice with an inactivating point mutation in the thrombin cleavage site of GPV (*Gp5^{kin/kin}*) were generated and analyzed in comparison with *Gp5^{-/-}* mice.

Methods: Platelet function was studied in a broad range of *in vitro* assays and *in vivo* models of hemostasis, thrombosis and thrombo-inflammatory brain infarction using *Gp5^{-/-}* and *Gp5^{kin/kin}* mice as single mutants or in combination with deficiencies of (hem)ITAM receptors, GPVI and/or CLEC-2 or critical components of their downstream signaling machinery.

Results: In agreement with previous reports, platelets from *Gp5^{-/-}* mice showed a slightly increased activation response to thrombin *in vitro* translating into a very mild acceleration of hemostasis and arterial thrombus formation. Remarkably, however, GPV deficiency completely restored defective hemostasis in mice with deficiencies in (hem)ITAM receptors or defects in their downstream signaling. Moreover, GPV-deficiency fully reverted protection of GPVI-deficient mice from arterial thrombus formation and brain infarction. Platelets from *Gp5^{kin/kin}* mice showed normal activation responses to prominent agonist, including thrombin, *in vitro*, but were completely resistant to GPV cleavage by the latter. Very unexpectedly, these animals reproduced the phenotype of *Gp5^{-/-}* mice in hemostasis and thrombosis in the presence as well as absence of a functional GPVI receptor.

Conclusion: These results reveal a central regulatory role of GPV in thrombosis and thrombo-inflammatory processes and indicate that its cleavage by thrombin is an essential step in this process.

Disclosure of Interest: None declared.

AS095

Platelet integrin alpha 6 beta 1 promotes metastatic disseminationMammadova-Bach E^{1,2,3}, Freund M^{1,2,3}, Bagnard D^{2,3,4}, Gachet C^{1,2,3} and Mangin P^{1,2,3}¹UMR_S949, Inserm, Etablissement Français du Sang-Alsace (EFS-Alsace); ²Université de Strasbourg; ³Fédération de Médecine Translationnelle de Strasbourg (FMTS); ⁴Inserm U1109, MN3T, LabEx Medalis, Strasbourg, France

Background: Cancer progression is a multistep process, where tumor cells acquire properties that enable their survival, proliferation and invasion, finally leading to dissemination and establishment of metastasis, which represents the major cause of cancer-related death. Platelets encounter tumor cells during their transit through the bloodstream. They are known to support metastasis by adhering to circulating tumor cells and providing a shield to allow immune escape, and actively induce a motile and invasive phenotype in cancer cells. Several platelet adhesion receptors were described to participate in metastatic dissemination, but the role of $\beta 1$ integrins, including $\alpha 6\beta 1$, the main receptor for laminins, is unknown.

Aims: To evaluate the role of platelet integrin $\alpha 6\beta 1$ in metastasis: **Methods:** Mice deficient for the platelet integrin $\alpha 6\beta 1$ (PF4-Cre- $\alpha 6^{-/-}$) and control (PF4-Cre- $\alpha 6^{+/+}$) mice were compared in experimental and spontaneous metastasis models induced by injection of B16F10 melanoma and MMTV-PyMT (mammary tumor virus-driven polyoma middle T) mammary tumor cells, respectively. Platelet adhesion to tumor cells was examined *in vitro* by quantification of platelet marker RAM-1 (GPIb β).

Results: Our results show that platelet integrin $\alpha 6\beta 1$ accelerates and increases lung colonization by B16F10 melanoma cells ($n = 6$; $**P < 0.01$; $***P < 0.001$). In an orthotopic MMTV-PyMT breast cancer model this integrin had no impact on primary tumorgrowth, but enhanced lung metastasis ($n = 6$; $**P < 0.01$). Antibody-mediated blockade of integrin $\alpha 6\beta 1$ in human platelets inhibited their adhesion to human breast cancer cells (MDA-MB-231, $n = 4$; $*P < 0.05$; SKRB-3, $n = 4$; $*P < 0.05$). Similar results were observed with murine platelets lacking integrin $\alpha 6\beta 1$.

Conclusion: This study demonstrates that platelet integrin $\alpha 6\beta 1$ contributes to metastatic process by mediating communication between the platelets and tumor cells. Targeting of platelet integrin $\alpha 6\beta 1$ could represent a novel therapeutic approach to prevent metastasis.

Disclosure of Interest: None declared.

AS096

Peptides inhibiting heparanase procoagulant activity significantly reduce tumor growth and vascularization in a mouse modelCrispel Y, Axelman E, Tatour M, Kogan I and Nadir Y
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Background: Heparanase is implicated in angiogenesis and tumor progression. Our previous studies demonstrated that heparanase might also affect the hemostatic system in a non-enzymatic manner. It forms a complex and augments the activity of the blood coagulation initiator tissue factor (TF). Peptides, developed by us, derived from TF pathway inhibitor (TFPI)-2 significantly reducing heparanase procoagulant activity were recently shown to attenuate inflammation in a sepsis mouse model (Axelman *et al.*, Thromb Haemost, 2014).

Aims: The current study was designed to assess the peptide effect on tumor growth and vascularization.

Methods: B16 mouse melanoma, EMT-6 mouse breast cancer, and MDA-231 human breast cancer cell lines were injected subcutaneously (s.c.). Inhibitory peptides 5, 6 and 7 were injected s.c. opposite to the tumor side, at a dose of 2–4 mg kg⁻¹, every other day, starting 4 days after tumor cell injection, for 3 weeks. To assess the effect on tumor relapse, the tumor was left to grow to 1–1.5 cm, and was extracted following peptide injections for 3 weeks. Tumor, organs, and plasma were studied.

Results: Peptides 5, 6 and 7 inhibited tumor growth and vascularization in the three cell lines in a dose-dependent manner, reaching a 2/3 reduction compared to control tumors ($P < 0.001$). Additionally, survival benefit ($P < 0.05$) and reduced plasma thrombin-antithrombin complex ($P < 0.05$) were observed in the treated groups. Peptides delayed tumor relapse by 6 days and reduced relapsed tumor size ($P < 0.001$). *In vitro*, peptides neither inhibited tumor cell proliferation nor affected heparanase enzymatic activity.

Conclusion: Peptides inhibiting heparanase procoagulant activity significantly impeded tumor growth, vascularization and relapse. The procoagulant domain in heparanase protein may play a role in tumor development, suggesting a new mechanism of coagulation system involvement in cancer.

Disclosure of Interest: None declared.

Non-vitamin K antagonist oral anticoagulants

AS097

Noacs in venous thromboembolism – strengths and caveats

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The most recent milestone on the road to improved treatment of venous thromboembolism (VTE) was the introduction of non-vitamin K antagonist oral anticoagulants (NOACs). They provide mainly a more convenient option without laboratory monitoring, easy discharge home, and simplified perioperative management. Extended duration therapy will be more acceptable for patients. However, without monitoring there is an increased risk of non-adherence, although preliminary studies have not demonstrated that. The risk of intracranial bleeds is reduced also in the VTE indication but minor gastrointestinal bleeds are more common. NOACs appear to be at least as effective as warfarin in patients with cancer and VTE but the investigated populations were selected. Further studies of NOACs vs. low-molecular-weight heparin are needed. For patients with thrombophilia, particularly anti-phospholipid syndrome, there have been a few case reports of NOAC failure. Conversely, data from case series and subgroup analyses do not confirm this suspicion. Management inpatients with renal failure is complicated by confusing messages from regulatory authorities in different jurisdictions. The management of VTE can be divided in 3 decision points at each of which there is a possibility to tailor the treatment according to the characteristics of the patient, (1) in the Emergency Room for those suitable for outpatient treatment, (2) at the time of discharge for hospitalized patients, and (3) after 3–6 months for possible extended duration of anticoagulation. The large phase III trials have answered questions regarding safety and efficacy. Many questions regarding details of management of patients with VTE remain to be properly addressed.

Disclosure of Interest: S. Schulman Grant/Research Support from: Boehringer Ingelheim, Octapharma, Baxter, Consultant for: Boehringer Ingelheim, Bayer, BMS.

AS098

Real life efficacy and safety of apixaban for stroke prevention in atrial fibrillation – results of the prospective noac registry (NCT01588119)

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Background: The management and outcome of long-term anticoagulation with non-VKA oral anticoagulants (NOACs) such as apixaban needs to be evaluated in daily care patients.

Aims: To prospectively evaluate effectiveness and safety of apixaban in consecutive atrial fibrillation (AF) patients treated with apixaban.

Methods: In the prospective, non-interventional Dresden NOAC registry a network of more than 230 physicians enroll eligible patients. Up to 3000 NOAC patients receive prospective follow up by phone visits by the registry office. All events are centrally adjudicated using standard scientific definitions.

Results: Until September 5th 2014, 511 patients receiving apixaban for atrial fibrillation were enrolled (60% male, mean age 74.8 years, mean CHADS₂-Score 2.0). 112 patients (21.9%) were switched from VKA to apixaban, mainly due to instable INR (29.5%) or bleeding (15.2%), the remaining patients were newly treated for AF. 41 patients (8%)

had a history of stroke or TIA. 64 patients (12.5%) had a history of malignant disease and 35 patients (6.8%) had a history of bleeding events. 78.9% of all patients received apixaban 5 mg bid, 20.5% received 2.5 mg bid; the remaining received non-approved dosages. During follow-up (median 355 days; 25th/75th percentile 184.5/494.5 days), the combined efficacy endpoint of stroke/TIA/systemic embolism occurred in 11 patients (2.53 per 100 patient years; 95% CI 1.26–4.52). Furthermore, 216 bleeding events occurred, of which 15 events were ISTH major bleeding (1.93 per 100 patient years; 95% CI 1.93–5.68). Overall, 8 patients died (1.80 per 100 patient years; 95% CI 0.78–3.56). 88.5% of patients were still taking apixaban at 12 month follow-up.

Conclusion: In daily care AF patients, apixaban demonstrates both high effectiveness and safety with outcome event rates in the range of the respective phase III results. Furthermore, treatment persistence with apixaban is high and much better than that reported for vitamin-K antagonists. Updated results will be presented at ISTH.

Disclosure of Interest: J. Beyer-Westendorf Grant/Research Support from: Pfizer, Boehringer Ingelheim, Bayer Healthcare, Daiichi Sankyo, Speaker Bureau of: Pfizer, Boehringer Ingelheim, Bayer Healthcare, Daiichi Sankyo, S. Werth Speaker Bureau of: Bayer Healthcare, L. Tittl: None Declared, F. Michalski: None Declared, S. Marten: None Declared, N. Weiss Grant/Research Support from: Pfizer, Boehringer Ingelheim, Bayer Healthcare, Daiichi Sankyo, Speaker Bureau of: Pfizer, Boehringer Ingelheim, Bayer Healthcare, Daiichi Sankyo.

AS099

Fixed-dose rivaroxaban is not associated with increased recurrent venous thromboembolism or major bleeding in patients with a high or low body weight

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Background: Rivaroxaban is a factor Xa inhibitor approved for the treatment of venous thromboembolism (VTE) using a fixed-dose regimen that is not adjusted for body weight, based on a similar efficacy and a significantly lower rate of major bleeding compared with standard therapy. Pharmacokinetic analyses showed a limited association between rivaroxaban exposure and body weight.

Aims: To determine the association between body weight and clinical outcomes during the use of rivaroxaban in comparison with standard therapy for treatment of VTE in the EINSTEIN DVT and PE studies. **Methods:** Oral rivaroxaban (15 mg twice daily for 3 weeks, followed by 20 mg once daily) was compared with subcutaneous enoxaparin followed by either warfarin or acenocoumarol for 3, 6 or

12 months in patients with acute symptomatic DVT and/or PE. Three groups of body weight were used: ≤ 50 , > 50 to < 100 and ≥ 100 kg. *P* values for trend were calculated for each of the treatment arms in relation to recurrent VTE and major bleeding.

Results: A total of 4142 rivaroxaban and 4129 standard therapy patients were eligible. Treatment characteristics did not differ substantially between the body weight categories. There was no association between body weight (≤ 50 kg [$n = 167$], > 50 to < 100 kg [$n = 6711$] and ≥ 100 kg [$n = 1393$]; ITT population) and risk of recurrent VTE: 6.7%, 1.9% and 2.3%, respectively, for patients on rivaroxaban ($P_{\text{trend}} = 0.54$) and 2.2%, 2.4% and 2.0%, respectively, for patients on standard therapy ($P_{\text{trend}} = 0.16$). Major bleeding showed no association with body weight for patients on rivaroxaban: 1.3%, 1.0% and 0.9%, respectively ($P_{\text{trend}} = 0.27$); however, major bleeding in patients on standard treatment was more abundant in patients with low body weight: 4.4%, 1.8% and 1.2%, respectively ($P_{\text{trend}} = 0.027$).

Conclusion: The fixed-dose rivaroxaban regimen for treatment of VTE is not associated with an increased risk for recurrent VTE in patients with a high body weight nor with an increased risk of major bleeding in patients with a low body weight.

Disclosure of Interest: M. Prins Grant/Research Support from: Bayer, Sanofi-Aventis, Boehringer Ingelheim, GSK, Daiichi Sankyo, LEO Pharma, ThromboGenics and Pfizer, M. Di Nisio Consultant for: Bayer and Grifols, M. C. Vedovati: None Declared, A. Riera-Mestre Grant/Research Support from: Bayer (TALENT program), Consultant for: Rovi, BMS, Pfizer and Daiichi Sankyo, K. Mueller Employee of: Bayer HealthCare, A. Cohen Consultant for: Bayer, BMS, Daiichi-Sankyo, Johnson & Johnson, Pfizer, Portola and Sanofi, P. Wells Grant/Research Support from: BMS and Pfizer, Consultant for: Bayer, Pfizer, Boehringer Ingelheim and Biomerieux, J. Beyer-Westendorf Consultant for: Bayer HealthCare, Boehringer Ingelheim, Daiichi Sankyo, Pfizer, Novartis and LEO Pharma, T. Brighton Consultant for: Bayer HealthCare, Boehringer Ingelheim, Daiichi Sankyo, Pfizer, Amgen Australia and GSK, H. Bounameaux Grant/Research Support from: Swiss National Foundation, Daiichi Sankyo and Bayer HealthCare, Consultant for: Pfizer and Bayer, J. Schneider Employee of: Bayer HealthCare, A. Lensing Employee of: Bayer HealthCare

AS100

Dabigatran persistence and adherence in New Zealand

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Background: Dabigatran was introduced in New Zealand in July 2011. It is free to patients and primarily used in atrial fibrillation. There is a perception that the lack of regular monitoring could adversely affect drug adherence.

Aims: To audit adherence in all patients on dabigatran in New Zealand over 3 years.

Methods: Data for all patients dispensed dabigatran from 1st July 2011 to 30th June 2014 were obtained from the National Pharmaceutical database which records all drugs dispensed in New Zealand. Ethics approval was obtained. The dose, number of tablets dispensed, date of dispensing, patient gender and age were collected. Adherence was assessed using the Medication Refill Adherence (total days' supply per days from first to last dispensing). 100% = sufficient tablets for time on treatment.

Results: A total of 30,205 patients filled at least 1 prescription; mean age 71.2 years, 59.6% male, 51.9% received 150 mg tabs. The mean age of patients taking 110 mg was higher than the 150 mg dose (77.7 years vs. 65.1 years). Medication persistence: 13% of patients filled only 1 prescription, 29% stopped treatment after 4 months, 55% continued treatment for > 12 months. These rates remained constant during the study period. 23,958 patients remained on treatment for > 30 days and were included in the adherence calculations. Drug adher-

ence was 100% in 64% of females and 55.7% of males during 1st 6 months of treatment. Adherence was related to age and drug dose (< 60 years – 43.3%, > 80 years – 65.7%; 110 mg – 62.8%, 150 mg – 55.5%; $P < 0.01$). Adherence decreased relative to the time on treatment (% with 100% adherence: Male: 6 m – 55.7%, 18 m – 40.1%, 30 m – 33.9%; Female: 6 m – 64%, 18 m – 51.8%, 30 m – 47%; $P < 0.01$).

Conclusion: This large study includes data from all patients taking dabigatran in New Zealand over 3 years. Adherence is high but declines significantly from 60% full adherence to 40% over 2 years. Younger patients on 150 mg dose have poorer adherence than older patients on the 110 mg dose. Regular review, especially in younger patients could potentially improve adherence.

Disclosure of Interest: P. Harper Shareholder of: INR Online Ltd (Warfarin Management Software), S. C. Fong: None Declared, L. Chen: None declared.

AS101

Increased risk of thrombotic events in patients with atrial fibrillation shortly after dabigatran or rivaroxaban discontinuation

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Background: Interruption of anticoagulant treatment with warfarin due to invasive procedures or bleeding represents a vulnerable period with increased risk of thromboembolic events. Less is known about the thromboembolic risk after discontinuation of non-vitamin K oral anticoagulants (NOAC).

Aims: Our aim was to systematically evaluate adverse events following temporary or permanent discontinuation of NOAC.

Methods: Data from a tertiary medical centre registry were analyzed regarding patients started on dabigatran or rivaroxaban from February 2012 to December 2013 with emphasis on thromboembolic events following temporary or permanent discontinuation of dabigatran or rivaroxaban.

Results: Among 866 patients started on NOAC, with a mean age of 74 (SD 8) years, and an average CHADS₂ score of 2.1 (SD 1.2), 705 patients were treated without interruption, while 84 patients had temporary interruption and 77 had permanent cessation of treatment. In patients without interruptions the rate of thromboembolic events was 1.0 (95% CI 0.4–2.1) per 100 patient-years, while in patients with interruption/cessation the rate of thromboembolic events was 21.6 (95% CI 10.3–45.2) per 100 patient-years, $P < 0.001$. There was a distinct clustering of thromboembolic events in the first weeks of NOAC discontinuation with the median occurring on day 14 (range 1–37 days) after discontinuation.

Conclusion: Dabigatran and rivaroxaban offer good protection against thromboembolic events during treatment, but discontinuation of NOAC increases thromboembolic risk more than 20-fold.

Disclosure of Interest: None declared.

Developmental hemostasis

AS102

Developmental haemostasis: consequences for clinical practice

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Hemostasis is a complex process leading to the formation of a blood clot at the site of vessel injury while simultaneously preventing excessive clotting. In the 1980's Maureen Andrew was the first to introduce the term 'developmental hemostasis' to describe the maturation of the hemostasis system from fetal to adult system. Significant differences exist in the physiology of primary hemostasis as well as secondary hemostasis and fibrinolysis in particularly fetal and neonatal life compared to adult life. The reasons for these differences are unclear and may be related to factors not associated with blood coagulation. In healthy neonates these differences are functionally balanced, as they do not cause bleeding or thrombosis. However, developmental hemostasis may have consequences for both the diagnosis and management of haemorrhagic or thrombotic events, especially in neonates.

Diagnosis of hemostatic disorders in neonates may be difficult as result of age-related changes in the coagulation system. Therefore, age-, analyzer- and reagent-specific reference ranges should always be used to avoid overdiagnosis and misdiagnosis.

Developmental haemostasis may be of importance in for example neonatal transfusion medicine. Regardless of reduced platelet function, bleeding time is shorter in neonates, probably as result of increased levels of VWF and high haematocrit. As *in vitro* studies revealed shorter closure times in neonatal blood transfused with adult platelets, adult platelets transfusion may increase the thrombosis risk, especially in preterm neonates. Furthermore, developmental haemostasis may influence the management of anticoagulation therapy in neonates as well. Decreased antithrombin levels may effect heparin treatment. Therapeutic levels of anti-Xa are difficult to obtain. Despite the delay in reaching therapeutic anti-FXa levels, thrombus resolution does occur. Age-specific guidelines for management of neonatal haemostasis is, therefore, warranted.

Disclosure of Interest: None declared.

AS103

Reduced dosing of enoxaparin to treat deep vein thrombosis (DVT) or pulmonary emboli (PE) in overweight and obese pediatric patients

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Background: The incidences of both DVT/PE and obesity have increased in the pediatric population. Minimal evidence addressing appropriate dosing in this population exists. Recent studies for adult obese patients suggest that lower enoxaparin dosing is adequate.

Aims: We sought to determine the adequacy of reduced dosing of enoxaparin in overweight and obese pediatric patients with DVT/PE in our institution.

Methods: We conducted a retrospective chart review of patients ≥ 12 years old with BMI ≥ 85 th percentile who were treated with enoxaparin for DVT/PE from January 2004 to December 2014. Patients with acute kidney injury, recent trauma or surgery, or increased risk of hemorrhage were excluded. Of 419 patients screened from our database, 30 met criteria. Data collected included demographics, enoxaparin dosing, anti-Xa levels, recurrent thrombosis/progression within

90 days, and bleeding events. We defined standard dosing (SD) as ≥ 0.90 mg kg⁻¹ q12 h. Using the binomial test, the recurrence/progression rates of the less than SD (<SD) and SD groups were compared to the 5% rate reported in the literature.

Results: In 30 patients (19 [63%] females, median age 15.5 years), the mean initial and final enoxaparin dose was 0.83 ± 0.14 and 0.77 ± 0.14 mg kg⁻¹ with mean initial and final anti-Xa levels of 0.70 ± 0.23 and 0.84 ± 0.19 units mL⁻¹ (therapeutic range 0.5–1.0 units mL⁻¹), respectively. Nineteen patients initially received <SD with mean dosing significantly lower than 11 who initially received SD (0.74 ± 0.10 vs. 0.98 ± 0.05 mg kg⁻¹, $P \leq 0.001$). The recurrence/progression rate of the <SD group (2/19, 10.5% [95% CI 1.3–33.1]) and the SD group (1/11, 9.1% [95% CI 0.2–41.3]) were not significantly different than 5% ($P = 0.245$, $P = 0.431$, respectively). After titration, 25/30 patients' final dosing was <SD.

Conclusion: In our small study, reduced enoxaparin dosing for overweight and obese pediatric patients with DVT/PE achieves therapeutic levels without an increased risk of thrombus recurrence/progression.

Disclosure of Interest: None declared.

AS104

Development of a rivaroxaban dosing regimen for treatment of VTE in children aged 12 to 18 years

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Background: Once-daily 20 mg rivaroxaban is efficacious and safe for the long-term treatment of VTE in adults.

Aims: To establish the rivaroxaban tablet dose in children aged 12–18 years that results in an equivalent exposure as observed after the 20 mg dose in young adults.

Methods: We conducted a phase I single-dose pharmacokinetic/pharmacodynamic (PK/PD) study followed by a 30-day multiple-dose PK/PD phase II study. A body weight-adjusted dose of once-daily rivaroxaban was derived using a physiologically based pharmacokinetic (PBPk) model. Predicted and observed plasma concentrations and corresponding exposures (AUC) were compared for the 10 and 20 mg equivalent doses in phase I and for the 20 mg equivalent dose in phase II. Additionally, the relationship of PD changes to observed plasma concentrations was evaluated.

Results: In phase I, observed plasma concentrations and exposures were in good agreement with PBPk model predictions. Comparison of exposure following the 10 mg ($n = 4$) and the 20 mg equivalent dose ($n = 5$) approximated dose linearity. In phase II, 11 children were included (mean age 15.5 years), and the observed plasma concentrations and exposures were in line with expectations and in line with prior phase I data. In both studies, the PT and aPTT showed a near-linear relationship with rivaroxaban plasma concentration. As a result, the rivaroxaban body-weight-adjusted dosing schedule is 15 mg and 20 mg, respectively, for children aged 12–18 years with a body weight of 30–50 kg and above 50 kg. No major bleeding occurred.

Conclusion: Phase I and II evaluations in children aged 12–18 years confirmed the rivaroxaban PBPk model predictions. These studies established a once-daily rivaroxaban dosing regimen that results in

similar plasma concentration–time profiles and exposure as observed in young adults treated with 20 mg rivaroxaban once daily. This dosing regimen is currently used for VTE treatment in children aged 12–18 years in phase III.

Disclosure of Interest: G. Young Consultant for: Bayer HealthCare, D. Kubitz Employee of: Bayer HealthCare, A. Chan Consultant for: Bayer HealthCare, G. Kenet Consultant for: Bayer HealthCare, C. Male: None Declared, P. Massicotte Consultant for: Bayer HealthCare, A. C. Molinari Consultant for: Bayer HealthCare, P. Monagle Consultant for: Bayer HealthCare, U. Nowak-Goettl Consultant for: Bayer HealthCare, R. Burghaus Employee of: Bayer HealthCare, J. Stampfuss Employee of: Bayer HealthCare, A. Lensing Employee of: Bayer HealthCare.

AS105

Developmental differences in fibrin clot formation under venous and arterial laminar flow

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Background: Infants and children have a significantly lower incidence of thrombosis compared with adults. Yet, the mechanism of blood clot formation, as the end product of coagulation, has not been studied.

Aims: To establish differences in the mechanism of fibrin clot formation during venous and arterial flow in infants and children compared with adults.

Methods: We adapted a plate and cone principle-based rheometer to study fibrin formation, while applying continuous laminar flow. Fibrin formation was studied at venous (300 s⁻¹) and arterial (800 s⁻¹) shear conditions. Viscosity plots were characterized by: base level (viscosity level before coagulation), lag time (time before increase in viscosity), plateau level (maximum viscosity), plateau-base level (increase in viscosity), time-to-plateau (TTP, time to reach the plateau level), and velocity (slope of the ascending curve between lag time and TTP).

Results: Fibrin clot formation was measured in triplicate in plasma samples of children aged 1 month to 1 year (*n* = 8), 1–5 years (*n* = 8), 6–10 years (*n* = 6), 11–16 years (*n* = 8) and adults (*n* = 8). Clot formation at venous shear rate was decreased in infants and children (Table). This difference was less pronounced at an arterial shear rate.

Conclusion: This is the first evidence that blood clot formation under physiologically relevant flow conditions is different children compared with adults and has major implications for treatment of thrombosis in children and adults.

Disclosure of Interest: None declared.

Alloimmune and autoimmune platelet disorders

AS106

Update on current attempts to prevent neonatal alloimmune thrombocytopenia

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Fetal/neonatal alloimmune thrombocytopenia (FNAIT) is often caused by maternal alloantibodies against the human platelet antigen (HPA)-1a, which opsonize fetal platelets. Subsequent platelet destruction is mediated via the Fc-part of the alloantibodies. The incidence of HPA-1a-mediated FNAIT in the Caucasian population is about 1 in 1500 live birth, based on a large population study, with no prophylactic measures to prevent maternal immunization. The most devastating risk of FNAIT is intracranial hemorrhage, which may lead to death or persistent neurological sequel in 10% of the clinically symptomatic cases. This makes antenatal management essential to avoid deleterious consequences. Currently, antenatal treatment options include intra-uterine platelet transfusion (IUT) to the fetus, or treatment of the pregnant mother with intravenous immunoglobulin (IVIG) with or without additional steroids. These treatment options have limitations, such as the risk of severe procedure-related complications causing iatrogenic fetal death, increase the risk for gestational diabetes and infection, and limited efficacy, respectively. As fetal platelet destruction is initiated after binding of maternal allo-antibodies to the fetal platelet surface, an attractive treatment option would be to block binding of maternal alloantibodies to the respective alloantigens on fetal platelets. In this overview, an update on current attempts to prevent FNAIT will be presented. Recent findings on the use of Fc-modified, murine as well as human monoclonal antibodies will be introduced. Moreover, results from a proof-of-concept study in a murine FNAIT model on the impact of anti-HPA-1a prophylaxis on the production of alloantibodies against platelets will be discussed. These studies support a new approach for minimally invasive treatment strategy for prevention of maternal immunization and fetal platelet destruction by maternal anti-HPA-1a alloantibodies.

Disclosure of Interest: None declared.

Age (years)	Base level (mPa.s)	Lag time (min)	Plateau level (mPa.s)	Time to plateau (min)	Plateau-base level (mPa.s)	Velocity index (mPa.s min ⁻¹)
< 1	1.25 ± 0.03***	1.38 ± 0.24**	2.41 ± 0.26*	2.59 ± 0.35*	1.16 ± 0.25*	1.61 ± 0.37*
1–5	1.31 ± 0.03	1.41 ± 0.24***	2.70 ± 0.36	2.67 ± 0.20**	1.39 ± 0.34	1.62 ± 0.45*
6–10	1.29 ± 0.04*	1.48 ± 0.21**	2.62 ± 0.15	2.74 ± 0.22*	1.33 ± 0.12	1.71 ± 0.31*
11–16	1.32 ± 0.06	1.40 ± 0.18**	2.65 ± 0.31	2.50 ± 0.28*	1.33 ± 0.26	2.24 ± 1.37
Adults	1.34 ± 0.03	1.00 ± 0.19	2.91 ± 0.34	2.21 ± 0.25	1.57 ± 0.33	2.17 ± 0.76

P* < 0.05, *P* < 0.005, ****P* < 0.001 compared to adults.

AS107

Impact of platelet activation detected by the washed platelet activation assay on thrombotic outcomes in patients clinically suspected of having heparin-induced thrombocytopenia

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Background: Heparin-induced thrombocytopenia (HIT) is caused by platelet (plt)-activating anti-plt factor 4 (PF4)/heparin (H) antibodies. Since HIT patients (pts) are highly prothrombotic for several days after onset, administration of an alternative anticoagulant soon after HIT has been clinically suspected is recommended. However, serum plt activating profiles vary across pts, which might affect outcomes.

Aims: The aim is to clarify the impact of plt activating properties on HIT-associated thromboembolic events (TEEs).

Methods: In a nationwide registry of pts clinically suspected of having HIT, 401 pts with results for an immunoassay for anti-PF4/H IgG and the plt activation assay were retrospectively included. For the latter, serum that activated washed plts only at a therapeutic H concentration, but not with anti-FcγRIIIa Abs, was defined as positive (pos). Among positive samples, serum that activated plts within 30 min or even in the absence of added H was defined as strongly pos. The remainder was considered weakly pos. We investigated the association between the results of serological assays and TEEs.

Results: The incidence of TEE in the pts who tested strongly pos, weakly pos, and negative (neg) in the activation assay were 71% (44/62), 47% (21/45), and 32% (95/294), respectively. The incidence of TEE in pts who tested neg in the activation assay but pos in the immunoassay was 32% (17/53), similar to that in pts who tested neg in both assays (32% [77/241]). On the day of clinical suspicion (defined as the day that the blood sample was drawn), 48% (30/62) of pts with strongly pos results did not have a TEE. However, TEE occurred in 40% (12/30) of these pts afterwards. In contrast, pts with weakly pos results showed a significantly lower incidence of TEE (11% [3/27]) afterwards ($P = 0.03$).

Conclusion: Stronger plt activation was significantly associated with a higher incidence of TEE even after the initiation of therapy. We may consider more intensive treatment strategies in pts with strong plt activation.

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AS108

Development and characterization of 5B9, a potential international standard for the diagnosis of heparin-induced thrombocytopenia

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Background: Heparin-induced thrombocytopenia (HIT) is a clinico-pathologic syndrome and its diagnosis is based on both clinical and biological criteria. However, despite recent improvements in HIT laboratory assay, a standard is always lacking.

Aims: The objective of our work was to evaluate whether a monoclonal humanized IgG1 against PF4/H complexes may serve for the standardization of HIT laboratory assays.

Methods: Transgenic GammaPrim[®] mice that directly produce chimeric antibodies with a human IgG1 Fc fragment were immunized with human PF4 and unfractionated heparin (UFH). After, fusion of splenocytes and subcloning, only one clone (5B9) producing an IgG1 that specifically bind PF4/H complexes without reactivity against PF4 alone was isolated.

Results: 5B9 was able to induce dose-dependent platelet activation in the presence of low concentration of UFH (0.5 IU mL⁻¹), and this effect not observed with high dose or without UFH, was fully inhibited by IV.3 antibody or dasatinib, an inhibitor of Src kinase. In addition, 5B9 increased TF mRNA expression after stimulation of isolated monocytes or whole blood in presence of UFH (0.5 IU mL⁻¹). Finally, 5B9 with UFH induced thrombocytopenia in transgenic mice expressing human PF4 and FcγRIIIa receptors.

5B9 was evaluated as a standard using 3 widely used laboratory immunoassays detecting anti-PF4/H (Stago, Hyphen Biomed) or anti-PF4/PVS (Immuco) antibodies, and the linearity (R^2) of standard curves obtained with these kits was always up to 0.95. Finally, we demonstrated using competitive immunoassays that 15 of 22 HIT plasma samples (68%) were able to inhibit (up to 20%) the binding of 5B9 to PF4/H complexes compared to 3/25 (12%) samples containing non pathogenic anti-PF4/H antibodies (no HIT).

Conclusion: 5B9 is the first anti-PF4/H monoclonal antibody with a human Fc fragment, and fully mimics the effect of HIT human antibodies. 5B9 is thus potentially useful for studying the physiopathology of HIT and for standardizing HIT biological assays.

Disclosure of Interest: None declared.

AS109

Different dosages of intravenous immunoglobulin (IVIg) in treating adult immune thrombocytopenia with long-term follow-up of 3 years: results of a case-control study including 91 cases

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Background: Immune thrombocytopenia (ITP), previously known as idiopathic thrombocytopenic purpura, is an acquired autoantibody-mediated disorder. The intravenous immunoglobulin (IVIg), as an expensive drug, is high cost-effective treatment measure for both of

children and adults patients with ITP. Some studies showed similar proportion of achieved a platelet count of more than $20 \times 10^9 \text{ L}^{-1}$ in both of the low-dose and high-dose IVIg group. We conducted a case-control study to explore the efficacy of different dose IVIg and the consumption of IVIg in ITP. Moreover, current study included adults patients with follow-up period reach 3 years.

Aims: The purpose of this study was to compare the effects of different dosages of intravenous immunoglobulin (IVIg) for treatment of immune thrombocytopenia.

Methods: A total of 91 eligible adults patients were included and followed-up 3 years in the case-control study. According to the different dosages of IVIg, patients with ITP were divided into three subgroups: group A (0.2 g kg^{-1} per day), group B (0.3 g kg^{-1} per day), group C (0.4 g kg^{-1} per day). Informed consents were obtained from all patients. The study was approved by the institutional review board of our hospital.

Results: In all of 91 adults with ITP, there was no significant difference of the therapeutic response among three groups of IVIg dosages ($P = 0.459$). The response rate of IVIg treatment in group A was 97.1%, and group B (97.2%), group C (100%). The mean time of platelet reach to $30 \times 10^9 \text{ L}^{-1}$ in group A was 2.5 days, group B (3.2 days), group C (2.9 days) ($P = 0.324$). The median IVIg consumption in group A was 0.83 g kg^{-1} , group B (1.22 g kg^{-1}), group C (1.64 g kg^{-1}) ($P = 0.000$). The results of follow-up showed no significant difference of clinical outcome among three groups of IVIg dosages.

Conclusion: The low-dose IVIg can be performed as effectively as high-dose regimen without increasing the rate of developing into chronic adults with ITP. Patients with ITP could get greater cost-effective from low-dose IVIg regimen.

Disclosure of Interest: None declared.

AS110

Higher or lower dose corticosteroids for primary immune thrombocytopenia: systematic review and meta-analysis

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Background: It is uncertain whether higher doses of corticosteroids, either up-front or cumulatively lead to better long term platelet count responses when treating primary immune thrombocytopenia (ITP).

Aims: To compare the effect of higher vs. lower dose corticosteroids on durable platelet count responses in patients with primary ITP.

Methods: We searched MEDLINE, EMBASE, CINAHL and the Cochrane Registry from 1970 to January 2015 and abstracts from the American Society of Hematology annual meetings from 2004 to 2014 for randomized controlled trials (RCTs) comparing platelet count responses in patients receiving different doses of corticosteroids for primary ITP. Higher and lower dose groups were defined by the initial dose received. We compared sustained platelet count responses (6 months after initial treatment) in each group. Secondary analyses were to compare groups based on cumulative corticosteroid exposure, short-term responses, and subgroups of adults and children. Estimates of effect were pooled using meta-analytic methods and a random effects model.

Results: We uncovered 2574 citations out of which we included 8 RCTs ($n = 1034$). Up-front higher dose corticosteroids were not associated with more frequent long or short-term platelet count responses (relative risk [RR] = 1.05, 95% confidence interval [CI] 0.79–1.40 and RR = 1.07, 95% CI 0.93–1.24, respectively). The effect was lost when high dose was defined by cumulative corticosteroid exposure. There were more frequent short-term complete responses (platelet count

$> 100 \times 10^9 \text{ L}^{-1}$) in the higher up-front dosing group (RR = 1.66, 95% CI 1.27–2.17; $P = 0.0002$). This effect was maintained within subgroups of adults and children. There were more corticosteroid related adverse effects in the lower up-front dosing group.

Conclusion: Higher dose corticosteroids were not associated with more frequent platelet count responses at 6 months but did result in more frequent short-term complete responses.

Disclosure of Interest: S. Mithoowani: None Declared, K. Gregory-Miller: None Declared, J. Goy: None Declared, M. Miller: None Declared, N. Noroozi: None Declared, D. Arnold Grant/Research Support from: GlaxoSmithKline, Amgen, Consultant for: Bristol-Myers Squibb, Amgen

Hemostasis and Infection

AS111

Hemostasis-pathogen interplay: the virus envelope

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Numerous virus types can affect hemostasis of which many are sheathed by an envelope, including HIV, dengue, Ebola, flu, cytomegalovirus, herpes simplex type 1 (HSV1) and more. Interestingly, the clinical correlations to hemostasis span a broad range from hemorrhage to occlusive vascular diseases, depending on the virus. The envelope consists of a phospholipid bilayer derived from the host cell and associated proteins encoded by both viral and host genes. Because the envelope is the virus surface, it is encountered first by the host, and is the pivotal trigger for infection, immune mobilization and pathology. We have been investigating the hypothesis that host cell- and virus-encoded envelope factors combine to facilitate activation of hemostatic proteases and these contribute to the infection process. As an envelope virus known to affect hemostasis, purified HSV1 has been our model. In support of the hypothesis, the physiological cell surface initiators of coagulation, tissue factor (TF) and anionic phospholipid, were integrated into the envelope. Using a novel panel of host and virus protein-restricted HSV1, clotting protease activation was shown to be dependent on the presence of envelope TF. Furthermore, the virus-encoded protein, gC, was found to enhance TF activity, which implies an evolutionary advantage to the virus. Both TF and gC on the virus increased *in vitro* infection through factor VIIa- and/or factor Xa-dependent stimulation of cellular protease activated receptor (PAR) 2. Thrombin also enhanced infection, but through PAR1. In a mouse model, TF-deficient HSV1 was remarkably less infectious than TF-positive virus. An anticoagulant TF antibody recognizing only viral antigen had a similar effect. These data suggest a general mechanism where the envelope of any virus propagated in a TF-bearing cell may initiate clotting enzyme activity thereby contributing to pathology and infection.

Disclosure of Interest: None declared.

AS112

Mutations in the control of virulence sensor gene from *Streptococcus pyogenes* after infection in mice lead to clonal bacterial variants with increased virulence

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Background: Group A *Streptococcus pyogenes* (GAS) is a gram-positive bacterium with the ability to disrupt host hemostasis, hijacking coagulation and fibrinolytic systems, thus allowing GAS to persist and

spread within a host. Many of the virulence factors necessary for this, including the human plasminogen activator, streptokinase, are regulated by the GAS control of virulence sensor/responder two-component system CovRS. Clones isolated from patients with invasive diseases often contain mutations in *covRS* genes leading to enhanced virulence. The mechanism of this regulatory phase shift that occurs during infection is poorly understood.

Aims: The goals of this study were to determine if mutations in the *covS* gene of animal-passaged isolates of GAS displayed any locational patterns, and to characterize *covS* mutated isolates for enhanced virulence.

Methods: The globally-disseminated MIT1 GAS, containing wild-type CovRS, was used and clones from wound sites, and/or the spleens, of infected transgenic mice containing human plasminogen, were isolated. Genes were subjected to DNA sequence analysis and cultures of GAS isolates were examined for a number of virulence factors including streptokinase.

Results: Several types of inactivating mutations were identified in all regions of the *covS* gene. The mutations ranged from single-nucleotide insertions and deletions to deletions of large segments of the gene. Many of the mutations resulted in early translation termination of the CovS protein. Strains with mutated *covS* displayed enhanced lethality and secreted several fold more active streptokinase.

Conclusion: Clonal variants with *covS* mutations exhibited high lethality and increased bacterial dissemination in a transgenic mouse containing human plasminogen. These data suggest that a randomly mutated *covS* gene occurs during the course of infection and is a strategy used by GAS to generate phenotypic heterogeneity.

Disclosure of Interest: None declared.

AS113

Secreted staphylococcal von Willebrand factor-binding protein binds to a sortase a-dependent factor to enable *S. aureus* binding to von Willebrand factor

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Background: The most frequent pathogen causing life-threatening endovascular infections and infective endocarditis is *Staphylococcus aureus* (*S. aureus*). Adhesion of *S. aureus* to blood vessels under shear stress requires von Willebrand factor (VWF). Staphylococcal von Willebrand factor-binding protein (vWbp) is a secreted protein that interacts with VWF fibers, enabling flow-controlled bacterial adhesion to endothelial cells and to subendothelial matrix via VWF. Surface proteins of *S. aureus* are positioned in the bacterial cell wall by a mechanism that involves cleavage of a conserved Leu-Pro-X-Thr-Gly (LPXTG) motif. A mutation in the *srtA* gene (surface protein sorting A or sortase A) leads to a defect in the anchoring of about 20 *S. aureus* surface proteins to the bacterial cell wall.

Aims: We hypothesized that in order for soluble vWbp to mediate bacterial adhesion to VWF, it has to interact simultaneously with VWF and with *S. aureus* itself.

Methods: We measured the adhesion of *S. aureus* Newman (WT) and mutants deficient in vWbp (*vwb*) or *srtA* (*srtA*) to VWF and vWbp respectively, in a micro parallel flow chamber by perfusing fluorescently labeled bacteria over a glass surface coated with VWF, collagen or vWbp. The contribution of exogenously produced vWbp added to the medium was analyzed during shear-dependent *S. aureus* adhesion.

Results: We found that exogenously produced vWbp restored *vwb* binding to VWF under shear stress. Likewise, compared to binding of the WT strain, the *vwb* strain was able to adhere to coated vWbp under shear stress, illustrating that both soluble and coated vWbp are able to interact with a surface receptor on *S. aureus*. In addition, soluble vWbp mediated *S. aureus* recruitment to glass-coated VWF. However, the *srtA* strain revealed impaired adhesion to vWbp or to VWF in flow.

Conclusion: We conclude that vWbp interacts both with sheared VWF and with a *srtA*-dependent *S. aureus* surface protein, mediating *S. aureus* binding to VWF. Further work will identify the *srtA*-dependent *S. aureus* protein.

Disclosure of Interest: None declared.

AS114

Minimal tissue factor expression reduces blood-brain barrier permeability and susceptibility to neurological symptoms in experimental cerebral malaria

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Background: Sequestration of *Plasmodium falciparum*-infected erythrocytes in the brain results in a severe neurological syndrome termed cerebral malaria (CM). Although inflammation, thrombosis and endothelial disruption are observed in CM, the extent to which coagulation is responsible for disease is incompletely understood.

Aims: To assess the role of Tissue Factor (TF) in blood brain barrier (BBB) disruption and the onset of neurological symptoms in experimental CM.

Methods: Mice transgenic for human TF expressed at 1% of the normal level (LTF^{-/-}), mTF heterozygous littermates (LTF^{+/-}) and TF-intact C57BL/6J (B6) mice were infected with *Plasmodium berghei* ANKA, a CM-inducing murine malaria. Mice were monitored daily for parasitemia and exhibition of neurological symptoms, and sacrificed between days 4 (ED4) and 6 (ED6) post-infection. TF procoagulant activity was measured in brains with a one-step clotting assay. To assess the extent and location of BBB disruption, mice were injected with Evan's blue dye and intensity of brain staining was quantified. Brain pathology was assessed in H&E-stained histological sections.

Results: The strains exhibited varying susceptibility to CM; 47% (B6), 100% (LTF^{+/-}) or 30% (LTF^{-/-}) of mice succumbed to CM on ED5, and 75% (B6) or 0% (LTF^{-/-}) succumbed on ED6. TF activity was elevated in brains of CM-positive (CM+) B6 (100-fold, ED5; 10-fold, ED6), LTF^{+/-} (1000-fold, ED5) and LTF^{-/-} (100-fold, ED5) mice vs. those with uncomplicated malaria. Though extensive hemorrhage was seen in CM+ B6 and LTF^{+/-} brain sections, minimal to no hemorrhage was seen in brains of CM+ LTF^{-/-} mice. Extensive Evan's blue staining was seen in brains of CM+ B6 and LTF^{+/-} mice; however, CM+ LTF^{-/-} brains showed minimal, focal staining.

Conclusion: Increased TF activity in brains of CM+ mice and reduced BBB permeability of LTF^{-/-} mice suggest TF is playing a significant role in CM pathogenesis. Ongoing studies are assessing mechanisms by which this occurs, with emphasis on thrombin-dependent PAR1 signaling.

Disclosure of Interest: None declared.

Endothelial cell wall + signaling

AS115

Engineering microvasculature and the organ-specific vascular niche

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The microvasculature defines the biological and physical characteristics of the surrounding tissue environment, and plays a role in the initiation and progression of many pathologies. Recapitulation of the microvasculature and an organ-specific vascular niche *in vitro* can provide a platform to study complex vascular phenomena, and organ-specific functions. In this talk, I will first present our work on engineering microvascular beds to uncover the biophysical requirements (flow and geometry) for initiating systemic microvascular thrombosis. I will then present our recent progress on reconstructing two specific vascular niches – in the kidney and the marrow respectively. To engineer a human kidney microvascular niche, we developed methods to isolate human kidney peritubular microvascular endothelial cells, and further reconstruct human kidney microvessels. These human kidney microvessels have distinct structure and barrier function, and uniquely respond to cyclosporine A, a calcineurin inhibitor, when compared to other endothelial cells. Using our reconstructed marrow vascular niche, we demonstrated that megakaryocytes, when embedded in the matrix, migrated and differentiated towards the microvessel, penetrated the endothelial cells, and released the platelets into the circulation. Stromal cells in the matrix also interacted with microvessels through physical contact as well as chemical modification of the endothelial cell phenotype, which lead to selective homing when hematopoietic cells including monocytes and HSCs were perfused through the microvessels. Our work demonstrates that both intrinsic properties of endothelial cells and their microenvironment are necessary to properly recapitulate the organ-specific structure and function of microvascular niches. These results provide evidence to support the broad potential of our platform to elucidate the complexities of vascular biology and pathophysiology in both a systemic and an organ-specific context.

Disclosure of Interest: None declared.

AS116

Inhibition of RhoA-RHO kinase pathway attenuates cocaine-induced microvascular endothelial cell dysfunction: studies under static and shear flow conditions

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Background: Cocaine use is a strong risk factor for vascular ischemic events. We have recently demonstrated that cocaine induces RhoA/Rho kinase (ROCK) pathway activation (Blood 2012; 120:2177) and a proadhesive phenotype in human umbilical endothelial cells under static conditions (Arterioscler Thromb Vasc Biol 2014;34:2439).

Aims: Since ROCK activation is dependent in part on the cell type, the main aim of this work was to extend our studies to human microvascular endothelial cells (HMVEC) under static and also shear flow conditions.

Methods: Cultured HMVEC were supplemented for 5 h with cocaine (10 μ M) or vehicle and then co-cultured with platelets (static adhesion) and immunostained for both, von Willebrand factor (VWF) and

GP1b. Experiments under flow conditions, were performed by perfusing calcein-labeled platelets over HMVEC monolayers cultured in a microfluidic flow chamber (Bioflux 200, Fluxion Biosciences). All the experiments were conducted in the presence or absence of Y-27632 (10 μ M), which target the catalytic domain of ROCK.

Results: HMVEC exposed to cocaine expressed significantly higher amount of VWF on the surface and adhered a larger number of platelets (13.3 ± 1.9 vs. 5.5 ± 1.5 platelets per cell, respectively; $P = 0.024$), compared with vehicle under static conditions. These phenomena were inhibited significantly by Y-27632 ($P < 0.05$). Under shear flow conditions, cocaine exposure of HMVEC resulted in 2.5-fold increase in surface VWF ($P < 0.05$) as well as a 3-fold increase in the number of platelets adhered and aggregated on the cell surface with respect to the vehicle-treated cells ($P < 0.001$); platelet adhesion was importantly reduced by co-incubation with Y-27632 ($P < 0.01$).

Conclusion: Our results show that in HMVEC cocaine induces a pro-adhesive phenotype particularly evident under shear flow condition. The inhibition exerted by Y-27632 suggests that activation of RhoA/ROCK pathway plays also a role in cocaine-induced microvascular endothelial cell damage.

Disclosure of Interest: None declared.

AS117

Mapping endothelial cell activation in the cardiovascular target organs using ultra-sensitive molecular magnetic resonance imaging

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Background: Endothelial activation is a hallmark of cardiovascular diseases, acting either as a cause or a consequence of organ injury. In particular, activated endothelial cells promote platelet activation and subsequent thrombosis. To date, we lack suitable methods to measure endothelial activation *in vivo*.

Aims: To develop a molecular imaging method to detect activated endothelial cells *in vivo*.

Methods: Magnetic resonance imaging (MRI) enhanced by a new formulation of microparticles of iron oxide targeted to activated endothelial cells.

Results: Thanks to the use of micron-sized superparamagnetic particles targeted to activated endothelial cells and the optimizations performed, the sensitivity of the method was dramatically higher than previously reported molecular MRI strategies. We were able, for the first time, to reveal single activated vessels in all cardiovascular target organs with an exceptional conspicuity. In clinically relevant contexts in mice (including sepsis, acute and chronic kidney diseases, diabetes mellitus and normal aging), we provided evidence that this method allows detecting endothelial activation before any clinical manifestation of organ failure in the brain, kidney and heart with an exceptional sensitivity. In particular, we demonstrated that diabetes mellitus induces chronic endothelial cells activation in the kidney and heart vessels. Moreover, aged mice presented activated endothelial cells in the kidneys and the cerebrovasculature.

Conclusion: These results demonstrate the feasibility of detecting silent endothelial activation occurring in conditions associated with high cardiovascular risk using an ultra-sensitive molecular MRI approach. This method allows taking a snapshot of the endothelial activation status in multiple organs at the same time and in a timely manner. Clinical translation of the present technology could help to identify and treat patients at high risk of thrombotic complications.

Disclosure of Interest: None declared.

AS118

Regulation of stimulated and basal release of weibel-palade bodies by syntaxin-3 containing snare-complexes

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Background: Endothelial cells contain unique secretory organelles, Weibel-Palade bodies (WPBs), which contain a number of hemostatic, angiogenic and inflammatory mediators. Their main component is the hemostatic glycoprotein VWF. Several components that are critical for regulated WPB exocytosis have been identified, including the small GTPase Rab27A and its effector Slp4-a, but the mechanism remains unclear. We have previously identified STXBP1 as an endogenous Slp4-a binding partner involved in WPB release, along with the SNARE proteins syntaxin-2 and -3.

Aims: In this study we investigated the possible role of syntaxin-2 and -3 in WPB exocytosis.

Methods: Intracellular localization of syntaxins was characterized by immunocytochemistry. Interactors of syntaxin-3 were identified by mass spectrometry. Syntaxin-3 was depleted using RNA interference.

Results: Syntaxin-2 was primarily associated with the plasma membrane where it localized at VE-cadherin-based adherence junctions and at integrin-based adhesions to the extracellular matrix. Interestingly, syntaxin-3 was primarily associated with WPBs. We mapped the endothelial interaction partners of syntaxin-3 through an unbiased mass spectrometry approach using pull downs of lentivirally expressed mEGFP-syntaxin-3 with anti-GFP nanobeads. Among its interaction partners are various SNAREs and associated proteins such as STXBP2 and STXBP5, NSF, SNAP23 and α -SNAP, suggesting we pulled down a SNARE complex and its regulatory machinery that are involved in exocytosis. After silencing of syntaxin-3 expression, we observed a decrease in basal (unstimulated) VWF secretion, which amounts to a significantly increased intracellular VWF content. This increase was related to a potentiation of Ca^{2+} - as well as cAMP-mediated VWF secretion. When corrected for increased WPB content the probability of stimulated release of a WPB was unaltered in the absence of syntaxin-3.

Conclusion: Our data position syntaxin-3 as a WPB-linked SNARE-protein that regulates basal secretion of VWF.

Disclosure of Interest: None declared.

FVIII

AS119

Regulation of factor VIII clearance: a multifactorial process

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The life cycle of factor VIII (FVIII) has been the subject of extensive investigation over the past three decades. In 2014, after prolonged debate, the predominant site of FVIII production was shown to be the vascular endothelium. Similarly, the fate of FVIII after its circulatory life span has been the focus of many studies. This information has acquired increasing clinical relevance, with the current attention to developing FVIII molecules with extended half-lives. The outcome of clinical trials with the first generation of these altered FVIII proteins has demonstrated that a half-life ceiling of ~1.5-fold, over the wild type molecule, is consistently achieved. This, in turn, has been demonstrated to represent the dominant effect of von Willebrand factor (VWF) on FVIII clearance. Thus, any extension of FVIII half-life

beyond 1.5-fold will need to overcome the regulatory influence of VWF. The influence of VWF on plasma FVIII levels has also been demonstrated in genome wide association studies.

Studies reported to date, indicate that the clearance of FVIII involves several different cell surface receptors, on a number of different cell types, and that FVIII is probably cleared both with and without VWF. Initial findings relating to FVIII clearance demonstrated a role for the low density lipoprotein receptor-related protein 1, LRP1. This receptor is expressed on the surface of macrophages and has been shown to bind to and clear both FVIII and VWF. Interestingly, the clearance of these ligands is enhanced under conditions of shear, suggesting that conformational changes are required to facilitate receptor interactions. Additional recent studies have focused on candidate clearance receptors identified in the CHARGE GWAS meta-analysis. These studies indicate that lectin and scavenger receptors expressed on liver sinusoidal endothelial cells (e.g. CLEC4M and stabilin-2) also contribute to the clearance of both VWF and FVIII.

Disclosure of Interest: None declared.

AS120

Enhanced factor VIII gene therapy for hemophilia a dogs with a novel Furin-evading factor VIII variant

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Background: Furin is an intracellular protein convertase system that is vital in several biological processes including coagulation. In the development of B-domain deleted (BDD) Factor VIII (FVIII) for hemophilia A (HA) therapy, a short sequence containing a putative Furin cleavage site was retained because it was assumed to be critical for intracellular processing and secretion, though this has never been demonstrated.

Aims: To determine the role of Furin in FVIII biology and develop improved therapeutics for HA.

Methods: We engineered a human FVIII variant where the Furin site was deleted (FVIII^{PF}). FVIII levels were determined with clotting assay and ELISA.

Results: FVIII^{PF} has both a 3-fold increased activity and secretion from BHK stably cells compared with FVIII^{BDD}. Conversely, intracellular FVIII^{PF} was lower. We observed similar results using furin-specific inhibitors in BHK-FVIII^{BDD} producing cells. Importantly, FVIII^{PF} levels were not affected by Furin inhibition. Furthermore, FVIII^{PF} and FVIII^{BDD} secretion from LoVo cells, a human line lacking functional Furin, were comparable. Infusions of recombinant canine FVIII^{PF} protein corrected the phenotype in naïve HA dogs. Furthermore, AAV8 liver gene therapy with cFVIII^{PF} in HA dogs at a log lower dose than previously used for cFVIII^{BDD}, resulted in therapeutic cFVIII levels of 2–3%. Upon protein challenges, no increased immunogenicity of cFVIII^{PF} was observed.

Conclusion: These *in vitro* and *in vivo* data suggest that Furin cleavage hampers FVIII biological activity. Our novel variant, FVIII^{PF}, exhibits enhanced secretion primarily by bypassing Furin cleavage and does not benefit from Furin inhibition. This variant exhibits improved hemostatic effects in both protein- and gene-based strategies. Thus these strategies have a strong rationale for translation to HA therapy.

Disclosure of Interest: None declared.

AS121

Swapping C-domains between factor VIII and V reveals a differential role of the two factor VIII C-domains in von Willebrand factor binding and intracellular trafficking to weibel-palade bodies in endothelial cells

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Background: We have previously shown that FVIII is co-targeted with von Willebrand Factor (vWF) to the Weibel-Palade bodies (WPBs) in blood outgrowth endothelial cells (BOECs), even when FVIII carries mutations in the light chain (domains *a3-A3-C1-C2*) that are associated with defective vWF-binding. This raises the question whether trafficking of FVIII is driven by multiple sites within FVIII, including the C1–C2 domains that are highly conserved in FV.

Aims: This study aims to dissect the role of individual FVIII C-domains in co-trafficking with vWF.

Methods: BOECs were transduced with lentiviral vectors encoding FV, YPF-tagged FVIII and FVIII/V-C1 and -C2 chimeras, and examined by confocal microscopy and subcellular fractionation. The same proteins were produced in 293-cells for functional studies. Structural implications of domain swaps were assessed by chemical foot printing and mass spectrometry.

Results: In contrast to FVIII, FV did not display targeting to WPBs. The FVIII/V-C1 and -C2 chimeras showed strongly reduced sorting to WPBs, suggesting that targeting requires the FVIII C1–C2 region. The purified FVIII/V C1-chimera lacked any vWF binding. In FX activation studies, this chimera proved functionally normal, except for some reduced affinity for FIXa. In contrast, FVIII/V-C2 proved similar to wild-type FVIII. This demonstrates that C1, but not C2, supports FVIII-specific functions such as assembly with vWF and FIXa. Foot-printing analysis revealed that both chimeras were more efficiently labelled than wild-type FVIII in their domain interfaces. These included the A1/C2 and the C1/C2 interface, suggesting that both chimeras display increased C2-domain mobility.

Conclusion: C-domain swapping between FVIII and V has limited effect on FVIII function, except for vWF binding. We propose that the disordered C2-domain orientation disrupts the natural tandem of the C-domain pair that is apparently required for co-trafficking with vWF in BOECs.

Disclosure of Interest: None Declared.

AS122

A population approach to hemophilia pharmacokinetics. Wapps: a web-service for bayesian post hoc estimation

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Background: The efficacy of factor VIII and IX concentrates administered to prevent bleeding episodes in patients with hemophilia A and B is correlated with the plasma levels measured over time after the infusion, reflecting the association between baseline factor level and spontaneous bleeding rate. The inter-patient variability of pharmacokinetic (PK) parameters is large, and assessing individual PK profiles is difficult due to the need for multiple samples. Population PK modeling provides a solution to this problem, but introduces complexity in the estimation process. The applicability of population PK modeling to factor concentrate administration in hemophilia patients has been repeatedly demonstrated, and some of the relevant covariates identified.

Aims: To develop a web accessible tool for reduced data individual PK estimation in hemophilia (www.wapps-hemo.org)

Methods: The study protocol has been registered on clinicaltrials.gov (NCT02061072) and waived the need for medical device exemption by FDA. Individual PK estimates are performed with NONMEM (ICON plc, Dublin, Ireland) and expert validated before release. The network collectively owns the database, which is available for additional research projects proposed and conducted by the members.

Results: We have developed a Web application allowing hemophilia treaters to obtain individual PK estimates on 2–4 data points after one or more infusions of any factor concentrate. Over 485 individual PK assessments from independent researchers and factor concentrate manufacturers have been used for modelling. The project is being piloted within a research network of 24 centers. The software architecture development, its testing and the population PK model development phases have been completed. We are prospectively testing the reliability of Bayesian PK post-hoc estimation and measuring its impact on clinical practice.

Conclusion: WAPPS has been successfully implemented and is available for field clinical testing. Interested new centers are invited to join.

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Platelet activation

AS123

Regulation of platelet membrane trafficking

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Activated platelets release hundreds of molecules that affect hemostasis, angiogenesis, inflammation, and wound healing. These molecules are stored in three granule types: dense, alpha, and lysosomes, and their release requires SNAREs (Soluble NSF Attachment Protein Receptors). v-SNAREs/VAMPs, on granules, pair with heterodimeric t-SNAREs (Syntaxins and SNAP-23/25s), on the plasma membrane, to mediate membrane fusion. Using platelets from knockout mice and from patients with Familial Hemophagocytic Lymphohistiocytosis (FHL), we are defining the platelet secretory machinery. Platelets from patients lacking Syntaxin-11 show a robust loss of secretion from all three granules. Deletion of VAMP-8, in mouse platelets, attenuates release; however deletion of VAMP-2, -3, and -8 is required for robust defects in hemostasis. SNARE regulators control where, when, and how SNAREs interact. Munc18b is a Syntaxin-11 chaperone required for secretion. Syntaxin Binding Protein 5/Tomosyn-1 regulates VAMP access to t-SNARE heterodimers. Munc13-4 is a docking factor that is especially important for dense granule release. SNARE post-translational modifications also affect function. Phosphorylation of SNAP-23 by I  B Kinase (IKK) controls SNARE complex formation and secretion. Acylation of SNAP-23 and Syntaxin-11 is critical for secretion. From our studies of platelet SNAREs, we have gained insights into other platelet membrane trafficking events, such as endocytosis. VAMP-3, Syntaxin-2/4, as well as ADP-Ribosylation Factor 6 (Arf6), contribute to fibrinogen uptake by circulating platelet. Interestingly, platelet endocytosis is also important for platelet spreading and for their response to certain Toll-like Receptor (TLR) agonists. Our studies of platelet secretion have led to a greater understanding of other forms of membrane trafficking in platelets and have suggested how these dynamic processes contribute to platelet function in circulation. Supported by NIH grant HL56652.

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AS124

Impaired nuclear accumulation and DNA binding of two FLI1 variants results in abnormally large platelet alpha granules and a reduction in platelet dense granule number

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Background: FLI1 is a transcription factor that is required for megakaryopoiesis. Patients with Paris-Trousseau Syndrome (PTS), who are hemizygous for *FLI1*, have an increased bleeding tendency, which is characterised by the presence, in platelets, of giant α -granules. We recently identified two mutations, p.R337W and p.Y343C, affecting residues in the DNA binding domain of FLI1 in members of two families with a bleeding diathesis, and reduced dense granule secretion and showed that they abolished transcriptional activity of FLI1.

Aims: To further characterise the effects of the R337W and Y343C variants on FLI1 function and granule morphology.

Methods: Electron microscopic (EM) analysis of platelets from heterozygous carriers of the *FLI1* defects was used to assess granule morphology. The effect of the variants on nuclear accumulation and DNA-binding was investigated by fluorescence microscopy and electrophoretic mobility shift assay, respectively, following transfection in Hek293 cells.

Results: EM analysis of platelets from patients with the *FLI1* defects revealed the presence of giant α -granules in 15% of sections examined and a 50% reduction in dense granule number. The R337W variant showed a 36% reduction in nuclear accumulation ($P < 0.0001$) compared with wild-type FLI1, while a reduction of over 80% in nuclear staining was observed for the Y343C variant ($P < 0.0001$). Compared to WT FLI1, and after correction for the amount of FLI1 in the nucleus, the R337W variant showed a 50% reduction in DNA-binding ($P < 0.05$), while binding of the Y343C variant was unaffected.

Conclusion: The loss in transcriptional activity of the R337W variant is due to the combined reduction in nuclear accumulation and DNA-binding capacity, while the Y343C variant fails to activate transcription as a result of impaired nuclear translocation. The presence of giant α -granules in platelets from patients with the R337W and Y343C variants suggests that loss of *FLI1* is the underlying cause of the abnormal α -granule morphology in patients with PTS.

Disclosure of Interest: None declared.

AS125

Dysregulation of pldn (Pallidin) as a mechanism for platelet dense granule deficiency in RUNX1 haplo deficiency

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Background: RUNX1 haplo deficiency is associated with thrombocytopenia and platelet dysfunction. Dense granule (DG) deficiency is reported in patients with RUNX1 haplo deficiency, but mechanisms are unknown. Platelet mRNA expression profiling in our patient with RUNX1 mutation showed downregulation of several genes (Sun *et al.* 2007 JTH 5: 146–154), including *PLDN* (fold change 0.239, $P = 0.029$; validated by qPCR). *PLDN* encodes for pallidin, a subunit of BLOC-1

(biogenesis of lysosome-related organelles complex-1) involved in granule/vesicle biogenesis. Pallid mice missing *pldn* and patients with *PLDN* mutations have DG deficiency.

Aims: We postulated that *PLDN* is a RUNX1 target and that its decreased expression contributes to the platelet DG deficiency in RUNX1 haploinsufficiency.

Methods: We studied RUNX1 regulation of *PLDN* in PMA (phorbol ester)-treated human erythroleukemia (HEL) cells. RUNX1 binding to *PLDN* was studied using chromatin immunoprecipitation (ChIP) and EMSA using HEL cell nuclear extracts. Luciferase reporter assays were performed.

Results: There are 6 RUNX1 consensus binding sites in 2288 bp of *PLDN* 5' upstream region. ChIP assay showed RUNX1 binding to *PLDN* chromatin at regions encompassing RUNX1 binding sites 1 (−184/−179 bp), 2 (−246/241 bp), 3 (−1370/−1365 bp), 4 (−1689/−1684 bp), 5 (−2065/−2060 bp), and 6 (−2242/−2237 bp). EMSA showed RUNX1 binding to all 6 sites. In luciferase reporter studies using constructs with 2288 bp of *PLDN* promoter, mutation individually of RUNX1 sites 1–6, except site 5, reduced activity by 60–70%. RUNX1 downregulation with siRNA decreased RUNX1 and *PLDN* protein expression. RUNX1 over-expression enhanced *PLDN* promoter activity and protein. Syntaxin-13, a t-SNARE, is a binding partner of *PLDN*; consistent with this, RUNX1 over-expression increased syntaxin-13 with increase in *PLDN*.

Conclusion: *PLDN* is direct target of RUNX1. These studies provide a potential mechanism for platelet dense granule deficiency in patients with RUNX1 mutations.

Disclosure of Interest: None declared.

AS126

Granuphilin (synaptotagmin-like protein 4, SLP4) regulates platelet dense granule secretion and aggregation

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Background: An increase in cytosolic calcium (Ca^{2+}) is a key signal contributing to platelet activation, yet the molecular mechanism by which it regulates secretion is unknown. Munc13-4, with two Ca^{2+} -binding C2 domains, may play a role as a Ca^{2+} sensor in platelets, but residual granule secretion present in *Munc13-4*^{−/−} platelets suggests a secondary mechanism. Granuphilin (SLP4), another protein with Ca^{2+} sensing C2 domains, was recently shown to contribute to Ca^{2+} -dependent secretion in a permeabilized platelet system. It was now important to determine its molecular role in regulating platelet secretion and function in response to agonist stimulation.

Aims: To characterize the role of granuphilin in platelets.

Methods: Granuphilin global knockout mice (*Slp4*^{−/−}) were used for analysis of platelet granule secretion and function. Interactions with SNAREs were identified in human platelets.

Results: We found an activation-independent granuphilin-syntaxin 11 interaction and confirmed an interaction with Rab8 also to be activation independent. *Slp4*^{−/−} platelets had a significant defect in 5-HT secretion in response to intermediate concentrations of thrombin while dense granule 5-HT loading was found to be the same between genotypes. Aggregation in response to the same concentrations of thrombin was also significantly reduced in *Slp4*^{−/−}, while integrin activation remained unchanged. P-selectin expression (α -granule marker) in response to thrombin was not affected. The levels of expression of critical SNAREs were not affected in *Slp4*^{−/−} platelets, confirming granuphilin deletion as the primary cause of the defects.

Conclusion: Granuphilin is specifically involved in dense granule secretion and in turn in platelet aggregation. This is in line with the potent role of the dense granule cargo ADP in augmenting platelet function. Better understanding of the mechanisms regulating dense granule secretion is important to develop successful antithrombotics without the detrimental side effects of current potent P2Y₁₂ inhibitors.

Disclosure of Interest: None Declared.

Platelet function testing assays

AS127

Can platelet function testing tell whether your patient will bleed or thrombose?

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Most platelet function assays have been developed for the diagnosis and management of patients presenting with bleeding rather than thrombosis. Notwithstanding, platelet function assays are increasingly being used for monitoring of the efficacy of antiplatelet drugs, with the aim of predicting which patients are likely to present with recurrent thrombotic complications or bleeding. Although current guidelines advise against routine monitoring of antiplatelet therapy, there is increasing evidence that platelet function assay results do indeed correlate with clinical outcomes in patients on dual antiplatelet therapy. Whether platelet function assessment provides simply a marker of worse prognosis or is a modifiable risk factor is a matter of debate. Numerous pharmacological studies support the benefit of adjusting antiplatelet therapy based on platelet function assays. However, several large randomized clinical trials have resulted in disappointment as platelet function assay-based individualization of therapy was not shown to be superior to standard therapy. This is in stark contrast to smaller studies of tailored antiplatelet therapy, where intensifying antiplatelet therapy in patients with insufficient platelet inhibition has resulted in fewer ischemic events, with no excess of bleeding complications. In the context of more potent antiplatelet agents clinically recommended as first-line therapy, the question of utility of platelet function testing is evermore present, with advocates of testing promoting tailoring of the intensity of antiplatelet therapy to individual patient needs, while opponents question the clinical benefits and economic value of such approaches. In this presentation, the pros and cons of platelet function testing as a predictor of future clinical events will be reviewed.

Disclosure of Interest: None declared.

AS128

Uncovering genetic networks underlying platelet response to thienopyridines by exome sequencing of clopidogrel-treated patients exhibiting extreme platelet reactivity

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Background: Platelet reactivity (PR) in patients treated with clopidogrel or other P2Y₁₂ inhibitors is variable and prognostic of clinical events. The genetic determinants of this PR phenotype play an important role (heritability, $h^2 = 0.73$) but previous GWAS approaches have identified just a few variants, explaining only 5–12% of PR variability.

New approaches, relying on additional genetic information, may identify an enlarged set of genes and pathways involved in PR.

Aims: To use whole exome sequencing (WES) to identify genomic variants that contribute to the variability of PR observed in response to clopidogrel treatment.

Methods: Ninety-six extreme PR phenotype patients adjusted for relevant clinical factors were selected from a cohort of 534 clopidogrel-treated patients (low PR with VASP = 26% vs. high PR with VASP = 77%). Compliance to clopidogrel was confirmed by the quantification of clopidogrel carboxylic acid in the serum. Average coverage of the exome bait region was 70-fold, with more than 98% of bases having at least 10× read-depth coverage.

Results: The exome analysis of 48 LPR vs. 48 HPR samples yielded 585 variants in 418 genes with a different frequency between LPR and HPR patients (adjusted $P < 0.05$). Principal component analysis showed complete discrimination of the two extreme PR groups, which was not dependent on the known CYP2C19*2 variant. A discriminant analysis of principal components showed that a minimum number of 130 variants were required to distinguish the two groups, and constituted a nominal gene-set for cross-validation. A pathway analysis of the top 130 variants revealed several genes involved in drug/xenobiotic metabolism. Additional variants of potential interest include those involved in actin cytoskeleton and also in in peroxisome transport function, lipid metabolism and ATPase activity

Conclusion: Our WES approach identified new candidate genes and pathways to further explain the high variability of the response to clopidogrel.

Disclosure of Interest: None declared.

AS129

Development of a small volume, whole blood, platelet function assay to assess neonatal platelet adhesion, activation, and aggregation

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Background: Neonatal platelets are considered hypofunctional compared to adult platelets. Current platelet function assays require large volumes of blood and are unreliable. The lack of functional assays that can be used with neonates hinders the evaluation of platelets in this population as well as the understanding of physiological differences between neonatal and adult platelets.

Aims: To develop a small volume, whole blood assay to evaluate neonatal platelet adhesion, activation, and aggregation.

Methods: To assess platelet activation, citrated whole blood was incubated with a P-selectin antibody and the platelet agonists TRAP-6, ADP, U46619, collagen-related peptide, epinephrine, or ristocetin. To evaluate platelet aggregation, citrated blood was divided, incubated with a FITC- or eFluor450-CD31 antibody, combined, and exposed to agonists. Double-labeled events and P-selectin expression were assessed with FACS. To assess static adhesion, citrated blood was exposed to agonists and incubated on VWF- or collagen-coated surfaces. To assess aggregation under shear, recalcified or citrated blood was perfused through collagen- or VWF-coated μ -chambers at 200 or 1500 s⁻¹. In select studies, blood was pre-incubated with a GPIIb/IIIa inhibitor or a GPIb blocking antibody.

Results: We first established the parameters for adult platelet function in response to a range of agonist concentrations. GPIIb/IIIa inhibition resulted in no platelet aggregation in response to all agonists except ristocetin and only a platelet monolayer on VWF and collagen surfaces. Blocking GPIb did not affect aggregation but eliminated ristocetin-induced agglutination. The assays performed require no more than 1 mL of blood as opposed to conventional platelet assays that require up to 20 mL.

Conclusion: We have developed a series of platelet function assays that evaluate platelet adhesion, activation, and aggregation. This assay will be integral in fully defining the functional phenotype of neonatal platelets in order to guide clinical practice.

Disclosure of Interest: None declared.

AS130

Platelet aggregation assays do not reliably diagnose platelet storage pool deficiency

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Background: Patients presenting with a significant variety of mucocutaneous bleeding symptoms including easy bruising, frequent epistaxis, bleeding gums upon tooth brushing and for women, heavy menstrual bleeding, are symptoms suggestive of a platelet disorder. Staple laboratory assays to evaluate platelet function include the Platelet Function Analyzer (PFA) and platelet aggregation analyses; both are known to have a number of limitations. Platelet storage pool deficiency (SPD) is a disorder related to decreased numbers of platelet granules. Platelet δ -SPD has been reported to have a decreased response to low levels of the agonist ADP in the second wave of light transmittance with platelet aggregometers or decreased ADP secretion by fluorescence luminometry. Two reports have described normal aggregation assays in 20–23% of patients having δ -SPD; these studies investigated only the response to the agonist ADP.

Aims: The purpose of our study was intended to evaluate laboratory aggregation test results for five agonists in patients diagnosed with platelet delta granule storage pool deficiency.

Methods: Platelet (PL) dense granules (DG) were enumerated using established electron microscopy protocols. Cases were also evaluated by light transmittance aggregometry using five agonists: ADP, epinephrine, collagen, arachidonic acid, and ristocetin. ADP was used at two concentrations. To date we have included 120 patients having δ -SPD. Our protocol conforms to institutional review board standards.

Results: Our patients had a mean of 2.90 ± 0.15 DG/PL (normal = 4–6 DG/PL). 27.5% of our patients were found to have normal aggregation assays for all five agonists. Response to epinephrine was abnormal in 69.2% of our patients; ADP was also frequent at 59.2%. Ristocetin was abnormal in 15.8% and collagen and arachidonic acid abnormalities were less frequent at 10% and 11.7% respectively.

Conclusion: These data suggest that platelet δ -storage pool deficiency may not be diagnosed if relying upon light transmittance aggregometry.

Disclosure of Interest: None Declared.

Assay challenges

AS131

Laboratory diagnosis of VWD

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Von Willebrand disease (VWD), the most common inherited bleeding disorder, is a commonly considered clinical diagnosis, and as such requires accurate and efficient diagnostic testing. Unfortunately, given the complexity of von Willebrand factor (VWF), such testing does not at present exist. Total levels of VWF protein can be measured easily by the VWF antigen, but antigen levels give no insight into VWF function. The ristocetin cofactor assay, a surrogate for VWF-platelet GPIb interactions, is commonly used to measure VWF function in terms of platelet binding, but suffers from high variability and lack of physio-

logic relevance. Novel assays of VWF function, such as direct GPIb binding assays using gain-of-function VWF, are a promising replacement. Factor VIII levels should be measured in workup of VWD, since VWF serves as the carrier protein for factor VIII and this function can be affected in some types of VWD. Another function of VWF is to bind exposed collagen at sites of injury; this function can be measured by collagen binding assays but is not always performed in initial VWD workup. In addition, collagen binding studies are complicated by the fact that multiple vascular collagens exist, and VWD variants may differentially affect VWF-collagen interactions. The ability of VWF to multimerize is critical to its function in hemostasis. This function can be measured by either agarose gel electrophoresis to examine multimer distribution or by sensitive collagen binding assays. Genetic analysis is becoming more frequent, and sequence variations in specific VWF domains link to specific types of VWD, particularly for the type 2 variants. The highly polymorphic nature of VWF, however, precludes easy interpretation of novel variants. Large deletions have also been found in types 1 and 3 VWD, and may not be readily found by standard sequencing techniques. Advances in VWF testing will bring clarity to the challenging diagnosis of VWD.

Disclosure of Interest: None declared.

AS132

Validation of a next generation DNA sequencing panel for haemostatic and platelet disorders

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Background: Next Generation sequencing (NGS) is transforming the delivery of diagnostic molecular genetics. Gene panels are being utilised to analyse groups of related disorders and to extend diagnostic capability in comparison with previous Sanger sequencing.

Aims: To develop and validate a gene panel for diagnostic/research NGS analysis of haemostatic and platelet disorders.

Methods: SureDesign software (Agilent), genome browsers and locus-specific mutation databases were used to select clinically relevant areas of each gene and to design 120mer biotinylated cRNA baits for the larger haemostatic and platelet genes: *ADAMTS13*, *F5*, *F8*, *F9*, *F13A1*, *F13B*, *FGA*, *FGB*, *FGG*, *ITGA2B*, *ITGB3*, *MYH9* and *VWF*. *F9* was included for patients where haemophilia type is unclear. Eleven patients' DNA was re-analysed using NGS following written informed consent. 1–16 variants were identified/gene. Only genes associated with each patient's disorder were analysed.

Results: All 52 variants previously identified by Sanger sequencing in the 11 individuals analysed were confirmed by NGS. Sequence coverage of regions of interest was ≥ 30 fold. Of eight variants in the *VWF* pseudogene region, all were identified with no pseudogene detection. The panel is now available for diagnostic and research analysis, with an 8 week turnaround time. For routine analysis, polymorphic single nucleotide variants (SNV) are removed by the NGS data analysis pipeline, so only those not previously seen or are likely to be pathogenic are observed in result data.

Conclusion: NGS analysis provides a single laboratory workflow for analysis of gene panels for related disorders as well as for whole genomes and exomes. Data analysis can include a single gene, such as *ADAMTS13*, or ≥ 1 gene for disorders such as those affecting fibrinogen. Data interrogation can also include analysis of more than one gene, e.g. for patients where defects in *VWF* or in *F8* may be responsible for reduced FVIII:C level without any further laboratory analysis.

Disclosure of Interest: None declared.

AS133

Comparative analysis of standard and new von Willebrand assays in a well typed cohort of patients with von Willebrand disease

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Background: Von Willebrand Disease (VWD) is an inherited bleeding disorder caused by a quantitative (type 1 and 3) or qualitative (type 2) defect of von Willebrand factor (VWF). VWF:Activity/VWF:Ag ratios are used to distinguish type 1 from type 2.

Aims: To compare 3 different automated VWF activity assays.

Methods: BCVWF:RCO (Siemens), HemosILVWF:RCO (Instrumentation Laboratory IL) and InnovanceVWF:Ac (Siemens) assays were done on 186 samples of well typed VWD (incl. VWF multimer and genetic VWF analysis) patients. By. VWF:Ag was measured by HemosILVWF:Ag (IL).

Results: Comparing HemosIL[®] and Innovance[®] values (using a ratio 0.76–1.25 as denoting identical) 146/186 samples were comparable, 22/186 higher with Innovance[®] and 18/186 higher with HemosIL[®]. The VWF:Act/VWF:Ag ratios with the 3 kits were significantly different with HemosIL[®]/VWF:Ag > Innovance[®]/VWF:Ag > BCVWF:RCO/VWF:Ag. For a cut-off ratio of < 0.7 for type 2 VWD a sensitivity of 98% with a specificity of 49% was found for BCVWF:RCO/VWF:Ag ratio. Only 1 type 2 sample, a type 2B, had a ratio of > 0.7, while 51% of all type 1 samples were misclassified as type 2. The HemosIL/VWF:Ag ratio had a sensitivity of 88% with a specificity of 91% for type 2. Using this ratio 6/48 type 2, all 2A/IIIE (6/21), were missed. The Innovance[®]/VWF:Ag ratio had a sensitivity of 96% with a specificity of 81%. 2/48 type 2 (2/21 type 2A/IIIE) were wrongly classified as type 1.

Conclusion: In a well typed cohort of VWD patients clear differences were noted between the 3 assays for VWF:Act, which become more relevant when comparing the VWF:Act/VWF:Ag ratio. The BCVWF:RCO/VWF:Ag ratio has the highest sensitivity for type 2 VWD but a very low specificity. The other tests showed lower sensitivity with improved specificity, with InnovanceVWF:Ac/VWF:Ag providing the best balance. Interestingly, all missed cases of type 2 VWD with HemosILVWF:RCO and InnovanceVWF:Ac were type 2A/IIIE (mutation D3 domain) while 2A/IIA were all picked up.

Disclosure of Interest: None declared.

AS134

ELISA-based assessment of excessive von Willebrand factor proteolysis

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Background: Von Willebrand disease-type 2A (VWD-2A) and acquired von Willebrand syndrome (AVWS) secondary to aortic stenosis (AS) or left ventricular assist device (LVAD) therapy are associated with an increased proteolysis of VWF. Electrophoresis-based assays represent currently the most sensitive tools to assess such increased VWF proteolysis. However, they have drawbacks impeding their large diffusion among laboratories, making thus challenging an early diagnosis and potentially differing appropriate medical care

Aims: To develop and evaluate an ELISA for the diagnosis of constitutive or acquired VWD associated with increased VWF proteolysis.

Methods: Our ELISA was assessed successively on samples obtained after perfusing whole blood from healthy donors in a HeartMate-II® (HM-II) LVAD mock circulatory loop model, in VWD-patients selected from the french cohort multicentric database CRMW ($n = 91$; detail: VWD-2A(IIA), $n = 30$; VWD-2B, $n = 25$; VWD-2A(III), $n = 20$; VWD-2M, $n = 14$; VWD-3, $n = 2$), in AVWS-patients ($n = 27$; detail: AS-patients, $n = 11$; HM-II patients, $n = 9$; and MGUS IgG - patients, $n = 7$) and in healthy subjects used as controls ($n = 33$). VWF proteolysis rate was expressed as a relative percentage with a standard of partially proteolyzed VWF specifically developed for this purpose.

Results: A speed-dependent increase of VWF proteolysis was assessed by ELISA after perfusing whole blood in the LVAD-model *in-vitro*. In VWD-patients, VWF proteolysis rate was significantly increased in VWD-2A(IIA) and VWD-2B, and significantly decreased in VWD-2A(III) vs. controls ($P < 0.0001$). In AVWS-patients, VWF proteolysis was significantly increased in AS- and LVAD-patients compared to controls ($P < 0.0001$) and no detectable in MGUS-IgG patients. A significant increase in VWF proteolysis was detected as soon as 3 h after LVAD implantation vs. pre-implantation values ($P < 0.01$).

Conclusion: Our ELISA might represent an helpful tool for the diagnosis and management of constitutive or acquired VWD secondary to increased VWF proteolysis.

Disclosure of Interest: None declared.

Therapeutic management of venous thrombosis

AS136

Additional catheter-directed thrombolysis for high proximal deep vein thrombosis; 5 year results of a randomized controlled trial (the CaVenT study)

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Background: Despite treatment according to current guidelines, acute deep vein thrombosis (DVT) affecting the upper femoral vein and/or the iliac vein is associated with a high risk of post-thrombotic syndrome (PTS). Additional treatment with catheter-directed thrombolysis (CDT) enhances clot removal and seems to preserve venous competence, reduce venous obstruction, and the risk of PTS. The CaVenT study is the first multi-center RCT to evaluate long-term clinical efficacy of additional CDT compared to standard treatment alone. We have reported that CDT reduced the frequency of PTS from 56% to 41% after 24 months follow-up (Enden *et al.*, Lancet 2012; 379: 31–8).

Aims: To examine whether additional CDT reduces the frequency of PTS after 60 months following a high proximal DVT.

Methods: Patients with a first-time objectively verified DVT affecting the upper femoral vein and/or iliac vein were randomized to receive conventional therapy alone or to additional CDT. The frequency of PTS at 60 m follow-up was assessed with the Villalta scale. The study was approved by the regional ethical committee and written informed consent was obtained from all patients.

Results: A total of 209 patients were randomized during 2006–2009. Mean age was 51.6 years (SD 16.8 years), 66 (38%) were females, and mean duration of symptoms was 6.5 days (SD 16.8 days). 87 (49.4%) had involvement of the pelvic veins. After 60 months follow-up data on clinical status was available for 176 patients (89 controls, 87 CDT). Preliminary data showed that 37 patients (43%; 95% CI 33–53%) allocated additional CDT had PTS compared to 63 (71%; 95% CI 61–79%) in the control group ($P < 0.001$), including 4 in the CDT group and 1 in the control group with severe PTS. The absolute risk reduction for PTS was 28% (95% CI 14–42%). The number needed to treat was 4 (95% CI 2–7).

Conclusion: Follow-up after 60 months showed an additional benefit of CDT. From the primary follow-up after 24 months, the frequency of PTS increased 1.4% in the CDT group and 15.2% among the controls.

Disclosure of Interest: None declared.

AS137

Anticoagulant therapy for symptomatic distal deep vein thrombosis: the cactus randomized placebo-controlled trial

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Background: There is uncertainty around the need to treat patients with a distal deep vein thrombosis (DVT) with anticoagulant therapy. We designed a randomized placebo-controlled superiority trial of therapeutic doses of low-molecular-weight heparin (LMWH) vs. placebo.

Aims: We aimed to assess the superiority of full anticoagulation vs. placebo for distal DVT treatment.

Methods: Patients diagnosed with a first distal DVT were randomized via an electronic platform to receive subcutaneous injection of nadroparin (170 UI kg⁻¹) once-daily or placebo for 42 days. The primary outcome was the rate of blindly adjudicated symptomatic proximal DVT or pulmonary embolism (PE) at 42 days.

Results: A total of 259 patients (male 51%, mean age 52.8 ± 16.7 years) were included. 126 were assigned to nadroparin and 133 to placebo. 4 patients withdrew consent (2 in each arm), and 3 were lost to follow-up (1 in the placebo arm and 2 in the nadroparin arm). In the intention-to-treat analysis at day-42, the primary outcome occurred in 7 patients in the placebo arm, and in 4 patients in the nadroparin arm: 7/130, 5.4% vs. 4/122, 3.3%, absolute difference - 2.1% (95% CI -7.8 to 3.5), chi² test *P* value 0.54. There were 5 major or clinically relevant non-major bleeds, all in the nadroparin arm: 5/130, 4.1% vs. 0/122, 0.0% in the placebo arm, Fisher test *P*-value 0.03. In the per-protocol analysis at day-42, the primary outcome occurred in 7 patients in the placebo arm and in 4 patients in the nadroparin arm: 7/125, 5.6% vs. 4/111, 3.6%, absolute difference -2.0% (95% CI -7.9 to 4), Fisher test *P*-value 0.55.

Conclusion: In this first placebo-controlled randomized trial of full-dose anticoagulation for distal DVT, there was no statistically significant difference between groups in the risk of symptomatic proximal DVT or PE at day-42. The use of full-dose anticoagulation was associated with a significant increased risk of bleeding.

Disclosure of Interest: None declared.

AS138

Statin use and risk of recurrent venous thrombosis: results from the mega follow-up study

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Background: Whether statin use after a first venous thrombosis reduces the risk of recurrence is uncertain.

Aims: We examined the risk of recurrent venous thrombosis in statin users vs. non-users.

Methods: Patients with a first venous thrombosis, who did not use statins prior to study inclusion (i.e. at time of first venous thrombosis), were selected from the MEGA follow-up study. Information on statin use was obtained by linkage to the Dutch Foundation for Pharmaceutical Statistics register. Linkage with this register was successful in 54% of all patients (*n* = 2547) of whom 2394 patients did not use statin prior to baseline. Cox-regression models with statin-exposure as a time-dependent variable were used to estimate hazard ratios (HR) with 95% confidence intervals (CI95) for risk of recurrence. Adjustments were conducted using a multivariable model and a propensity score model.

Results: During a mean follow-up of 5.2 years, statin therapy was initiated in 233 (9.7%) patients. There were 347 definite recurrent venous thrombotic events, of which 16 occurred among statin users. Statin use was associated with 24% reduced risk of recurrence (HR 0.76, CI 95% 0.46–1.26) after adjustments for age and sex; with 18% reduced risk after multivariable adjustments (HR 0.82, CI 95% 0.52–1.31); and 11% reduced risk when adjustment for confounding was done using propensity score matching (HR 0.89, CI 95% 0.53–1.51). Statin use had no effect on recurrence in patients with an unprovoked first event (multivariable HR 1.04, CI 95% 0.56–1.92).

Conclusion: Our findings suggest that statins have a modest effect on the risk of recurrent venous thrombosis, of a magnitude that is unlikely to lead to clinical utility. While we took particular care to minimize bias and confounding, the causality of the association is still unsettled.

Disclosure of Interest: None declared.

AS139

Long-term follow-up of a prospective cohort of unprovoked venous thromboembolism (VTE) patients off anticoagulants-risk stratification evaluation (REVERSE)

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Background: To continue oral anticoagulants (OAC) after short-term therapy for unprovoked VTE is controversial. In 2007, we explored potential predictors of recurrent VTE and developed the 'MEN continue and HERDOO2' (women with 2 of the following: (1) Hyperpigmentation, Edema or Redness (HER) on exam in either leg, (2) VidasD-Dimer > 250, (3) Obesity-BMI > 30 or (4) Older age > 6 (5) clinical decision rule to identify low risk patients with unprovoked VTE who could safely discontinue OAC.

Aims: We sought to confirm the high risk of recurrent VTE in high risk patients and conversely the low risk in low risk women over longer term follow-up and explore predictors of long-term recurrent VTE.

Methods: Multi-centre ($n = 11$) prospective cohort study of first unprovoked VTE patients recruited between 2001 to 2006. Symptomatic suspected VTE during subsequent follow-up (up to April 2014) off of OAC was investigated with reference to baseline imaging and then independently adjudicated.

Results: Of 647 participants, mean age was 53% and 49% were female. During a mean 5.1 years of follow-up, 167/647 suspected VTE were adjudicated as recurrent VTE resulting with annual risk of recurrent VTE of 4.9% (95% CI 4.2–5.7). Men had an 7.4% (95% CI 6.1–9.0) annual risk of recurrent VTE. High risk women had an annual risk of recurrent VTE of 5.9% (95% CI 4.2–8.1). Low risk women (1 or 0 HERDOO points) had 1.1% (95% CI 0.6–2.0) annual risk of recurrent VTE. Men with HER had an annual risk of recurrent VTE of 10.6% (95% CI 7.9–13.9) and women with HER had an annual risk of recurrent VTE of 6.0% (95% CI 4.1–8.6).

Conclusion: Men and high risk women with unprovoked VTE should be considered for long-term OAC therapy given a high risk of recurrent VTE after > 5 years of follow-up. HER is a strong predictor of recurrent VTE. Women with a low HER DOO 2 score may be able to safely discontinue anticoagulants but we await the results of the REVERSE II study (Q4 2016) to validate this important finding.

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Membrane scrambling

AS140

Scrambling the membrane: a regulatory mechanism of platelet function?

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Scrambling of the platelet membrane phospholipids, resulting in phosphatidylserine (PS) exposure, can be achieved by in a calcium-dependent way by potent platelet agonists, or in a relative calcium-independent way by metabolic depletion, ageing (apoptosis) or injury (necrosis). *In vitro*, the PS exposure leads to coagulation factor (F)II, FV, FIX and FX binding and activation, and consequently to a potent stimulation of thrombin and fibrin generation. Recent findings can provide an explanation why this platelet procoagulant activity is restricted to and at the same time propagated at the sites of vascular damage and thrombus formation. Substantial evidence comes from research to the Scott syndrome, a congenital mild bleeding disorder, characterized by defective calcium-dependent exposure of PS in platelets and other blood cells, and linked to mutations in anoctamin-6 (ANO6 or TMEM16F). It has become clear that this anoctamin has many more functions than acting as a 'membrane scramblase'. Data will be discussed from Scott patients or mice with deficiencies in anoctamins, indicating how anoctamin 6 regulates: (i) platelet swelling and change to a translucent shape; (ii) microvesiculation; (iii) transmembrane ion fluxes; (iv) inactivation of adhesion receptors; and (v) platelet protein composition and (vi) intracellular protein cleavage. The questions will be raised: (i) if membrane phospholipid scrambling is an isolated platelet response, and (ii) if it is the primary role of anoctamin 6 *in vivo* to regulate this platelet response.

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AS141

Platelets maximise membrane surface area through coordinated ballooning and procoagulant spreading to amplify localised thrombin generation

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Background: Platelets are central to the process of haemostasis, rapidly aggregating at sites of blood vessel injury and acting as coagulation nidus sites. Upon interaction with sub-endothelial collagen, platelets are transformed into balloon-like structures as part of the haemostatic response. It however remained unclear, how and why platelets generate these structures.

Aims: To determine the physiological relevance and the cellular and molecular mechanisms underlying membrane ballooning in human platelets.

Methods: 4D live-cell immunofluorescence imaging and correlative light-electron microscopy.

Results: We showed that human platelets adherent to collagen are transformed into phosphatidylserine-exposing balloon-like structures with expansive macro/micro-vesiculate contact surfaces, by a process which we termed procoagulant-spreading. We reveal that ballooning is mechanistically and structurally distinct from membrane blebbing and involves dramatic disruption to the platelet microtubule cytoskeleton and increased membrane permeability. Unlike blebbing, procoagulant ballooning is irreversible and consequent upon Na⁺, Cl⁻ and water entry; and whereas the hydrostatic pressure required for bleb formation is fluid entry-independent, the rapid expansion and sustained phases of ballooning are driven by external osmotic pressure. Inhibition of actin polymerisation diminished procoagulant-spreading, but promoted ballooning. Inhibition of Na⁺, Cl⁻ or water entry impaired ballooning, procoagulant-spreading and localised thrombin generation. Finally, human Scott syndrome platelets, which lack expression of Ano-6, also showed a marked reduction in membrane ballooning; an effect replicated by the inhibition of calcium activated chloride channels in control platelets

Conclusion: Ballooning and procoagulant-spreading are important mechanisms for the platelet procoagulant response at wound sites, allowing marked amplification of localised coagulation and haemostasis.

Disclosure of Interest: None declared.

AS142

Hysteresis-like binding of coagulation factor X/XA to lipids protects from blood flow

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Background: Binding of factors X (fX) and Xa to activated platelets is important for the formation of prothrombinase and intrinsic tenase, respectively. The mechanism of this binding remains unclear.

Aims: Aims of this work was investigate interaction of X or fXa with the membrane of activated platelets or purified phospholipid membranes

Methods: Flow cytometry, surface plasmon resonance and mathematical modeling were used to investigate interaction of X or fXa with the membrane of activated platelets or purified phospholipid membranes. Confocal microscopy was used to study binding of fXa to platelets thrombi formed in flowing whole blood under shear conditions.

Results: In agreement with previous reports, fX/fXa binding to phospholipids and procoagulant activated platelets was high-affinity and calcium-dependent, but unexpectedly only partially reversible. Dissociation of fX(fXa) was a two-step process ending with a plateau that was up to 10-fold greater than expected from association binding studies. In other words, the overall binding process was hysteresis-like, demonstrating a kind of 'memory'. The same hysteresis-like kinetics was observed for annexin V, and the overall phenomenon was shown to be explained by trimerization of the protein monomers on the membrane. Examination of fXa binding to platelet aggregates formed in a parallel-plate flow chambers coated with collagen revealed that hysteresis phenomenon persisted in such realistic thrombi with naturally occurring fXa concentrations suggesting its physiological importance in preventing fXa wash-out by flow.

Conclusion: Binding of fX (fXa) to either purified phospholipid membranes or activated platelets (either washed or in thrombi) had hysteresis-like behavior, related to multimerization of coagulation factor monomers on the phospholipid surface.

Disclosure of Interest: None declared.

AS143

Novel peptide and peptidomimetic inhibitors of prothrombinase assembly on phosphatidylserine inhibit thrombin generation and fibrin formation

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Background: Phosphatidylserine (PS) is an anionic lipid that is typically sequestered to the inner, or cytoplasmic, leaflet of bilayer membranes and only exposed on the outer leaflet during activated processes such as apoptosis or clotting. PS promotes coagulation by interacting with and allowing the assembly of coagulation protein complexes, e.g., prothrombinase. We have developed small (< 4 kDa) peptide and peptidomimetic probes that preferentially bind membranes containing PS by targeting the membrane's shape and lipid composition. We hypothesize that these PS probes will block the assembly of coagulation protein complexes, providing a new tool for inhibiting thrombosis.

Aims: To 1) evaluate the effectiveness of these PS probes as antagonists of coagulation factor/lipid membrane association and 2) determine if this activity translates into changes in enzymatic activity *in vitro*.

Methods: We use surface plasmon resonance to directly evaluate the effects PS probes have on coagulation factor/PS binding. Effects on coagulation protein complex assembly and enzymatic activity are examined using modified prothrombinase and microfluidic flow assays.

Results: We find the PS probes directly block coagulation factor/PS binding by measuring reduced association of factor Xa to an activated platelet like-membrane surface when it is pre-treated with a PS probe. PS probes significantly inhibit the enzymatic activity of prothrombinase in the presence of activated platelets, isolated microparticles, and synthetic liposomes. In whole blood, PS probes block fibrin cross-link-

ing of adherent platelets under physiologic flow conditions. Peptidomimetic PS probes show equivalent activity and increased stability in serum.

Conclusion: We demonstrate that small PS probes can antagonize coagulation protein assembly on PS and that this significantly reduces enzymatic activity *in vitro*. Future work includes characterization of localization and activity *in vivo*. These PS probes may represent a novel approach to therapeutic anticoagulation.

Disclosure of Interest: None declared.

Modulators of fibrinolytic activity

AS144

Novel approaches to modulating fibrinolytic activity

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Fibrinolysis can be enhanced through plasminogen activators (e.g. t-PA). Their clinical use, however, is limited by low efficiency and life-threatening side-effects. Therefore, new therapeutic approaches have been explored to enhance (endogenous) fibrinolysis. The latter is triggered by plasminogen activators (PA) which convert plasminogen to plasmin. Initial cleavage of fibrin by plasmin displays C-terminal lysines on the fibrin surface, which serve as propagators for plasmin generation. Endogenous fibrinolysis is hampered either through inhibition of plasmin (e.g. alpha₂-antiplasmin), through inhibition of PAs (e.g. plasminogen activator inhibitor-1, PAI-1) or through modification of the fibrin surface (e.g. activated thrombin activatable fibrinolysis inhibitor, TAFIa). PAI-1 and TAFI are widely studied antifibrinolytic proteins and have been linked to various thrombotic disorders. PAI-1 is a serine protease inhibitor (SERPIN) whereas TAFIa is a carboxypeptidase. The antifibrinolytic activity of TAFIa is threshold-dependent with the threshold value being proportionate to the plasmin concentration, and therefore also indirectly linked to PAI-1. The concerted action of PAI-1 and TAFIa by which protection of the fibrin clot is provided, has led to the idea of dual targeting strategies. Development of antibody-engineered inhibitors against TAFI and PAI-1 have been shown to stimulate fibrinolysis efficiently. A heterodimer diabody, Db-TCK26D6x33H1F7, cross-reactive with human, mouse and rat PAI-1 and TAFI was developed and was demonstrated to enhance fibrinolysis in *in vitro* clot lysis and thromboelastometric experiments. Its profibrinolytic properties were further confirmed *in vivo* in mouse models of thromboplastin-induced thromboembolism and in various mouse models of stroke. Taken together all data demonstrate that dual targeting of PAI-1 and TAFI results in a pronounced profibrinolytic effect with superior properties to those of t-PA and minimal bleeding risks.

Disclosure of Interest: None declared.

AS145

Effect of DS-1040 on endogenous fibrinolysis and impact on bleeding time in rats

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Background: DS-1040 is a novel inhibitor of the activated form of thrombin-activatable fibrinolysis inhibitor (TAFIa) intended to be used for the treatment of thrombotic diseases.

Aims: We investigated if DS-1040 enhances endogenous fibrinolysis in a rat venous thromboembolism (VTE) model and if DS-1040 prolongs bleeding time in a rat tail bleeding model.

Methods: *VTE model:* Rats were treated with tissue factor to generate microthrombi in circulation. DS-1040 was intravenously injected just before initiation of tissue factor treatment and blood samples were collected at 25 min after the end of the tissue factor treatment and plasma D-dimer levels were determined as an indicator of fibrinolysis. *Tail bleeding model:* Rats were treated with DS-1040 or t-PA intravenously. The rat tail was cut with a razor 5 min after administration and the bleeding time was measured.

Results: DS-1040 increased plasma D-dimer levels in the VTE model in a dose-dependent manner and statistically significant increases were observed at 0.063 mg kg⁻¹ or more. In the tail bleeding model, DS-1040 did not prolong the bleeding time at doses of up to 30 mg kg⁻¹ (480-fold higher than the effective dose), while 5.2 mg kg⁻¹ of t-PA did.

Conclusion: DS-1040, a novel TAFIa inhibitor, enhances endogenous fibrinolysis without prolonging bleeding time, suggesting DS-1040 is a promising new thrombolysis enhancer for the treatment of thrombotic diseases.

Disclosure of Interest: K. Noguchi Employee of: Daiichi Sankyo Co., Ltd., N. Edo Employee of: Daiichi Sankyo Co., Ltd., Y. Ito Employee of: Daiichi Sankyo Co., Ltd., K. Yamaguchi Employee of: Daiichi Sankyo Co., Ltd.

AS146

A novel method to develop plasminogen activator inhibitor-1 antagonist as thrombolytic agent

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Background: Current thrombolytic agents (recombinant tissue plasminogen activator, rtPA, or its variants) are effective but can have severe bleeding or neurotoxicity side effects. Plasminogen activator inhibitor-1 (PAI-1) is the key negative regulator of the thrombolytic system inhibiting both urokinase and tPA. Inhibition of PAI-1 activity was shown to prevent thrombosis and accelerate fibrinolysis. However, a specific antagonist of PAI-1 is currently unavailable in clinical practice.

Aims: To develop a specific PAI-1 antagonist and evaluate its anti-thrombotic potential.

Methods: Active-site mutated urokinase (uPA-S195A) does not possess proteolytic activity, but can bind to PAI-1 and form a Michaelis complex, and thereby prevent the binding of PAI-1 to active plasminogen activators. A major challenge of using uPA-S195A as a PAI-1 antagonist is its relatively weak inhibition of PAI-1. We screened a panel of uPA-S195A variants with mutations around the active site and developed a potent PAI-1 antagonist (named 'PAI-trap'). Furthermore, we tested its fibrinolysis potential in human plasma, and evaluated its anti-thrombotic effect in a laser-induced thrombosis mouse model.

Results: PAI-trap specifically inhibits human PAI-1 with high potency ($K_d = 0.15$ nM), and promotes fibrinolysis in human plasma by inhibiting endogenous circulating and platelet PAI-1. In addition, it also inhibits murine PAI-1, allowing the evaluation in murine models. In a laser-induced thrombosis mouse model, PAI-trap reduces fibrin generation and inhibits platelet accumulation at the site of injury.

Conclusion: PAI-trap effectively suppresses fibrin generation and inhibits platelet accumulation, and is a promising thrombolytic agent. PAI-trap may be used together with rtPA to lower the side effects of rtPA through reducing its dose.

Disclosure of Interest: None declared.

AS147

Inhibition of PAI-1 accelerates venous thrombosis resolution in type II diabetic mice

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Background: Type II diabetes mellitus (DM) is thought to be a risk factor for thromboembolism (VTE). Elevated plasma levels of plasminogen activator inhibitor-1 (PAI-1) are associated with DM. However, the role of PAI-1 in the development of DM-associated VTE is poorly defined.

Aims: Our aim was to determine the role of PAI-1 in the DM-associated VTE, and to test the hypothesis that inhibiting PAI-1 promotes venous thrombosis resolution.

Methods: Thrombosis was induced in the inferior vena cava (IVC) and saphenous vein of mice. Diabetes mellitus was induced by feeding mice high fat diet (DM group). Plasma was analyzed for glucose, non-esterified fatty acids, and PAI-1. PAI-039, a specific, small molecule pharmacological inhibitor of PAI-1, was orally administered to mice (2 mg kg⁻¹ twice daily). The gene expression of PAI-1, MMP-2, 9, MT1-MMP, and VEGF in thrombus were assessed by real-time PCR.

Results: Thrombus size and collagen content were substantially larger at 3 days after IVC ligation in DM mice compared to NC mice ($P < 0.05$). Intrathrombotic PAI-1, MMP-2, MMP-9, MT1-MMP, and VEGF gene expression were significantly enhanced in DM mice compared to WT mice ($P < 0.01$). We also subjected DM and NC mice to FeCl₃ saphenous vein injury and measured the time required to form an occlusive thrombus. The mean time to occlusion in DM mice (322 ± 27 s, $n = 6$) was significantly shorter than that in NC mice (473 ± 66 s, $n = 6$, $P < 0.05$). The inhibition of PAI-1 by PAI-039 reduced thrombus size in IVC ligation model ($P < 0.05$) and delayed vein occlusion time ($P < 0.001$) compared with vehicle-treated controls. The expression of intrathrombotic MMP-9, MT1-MMP, and VEGF, but not MMP-2 was markedly increased by the treatment of PAI-039 compared with vehicle group.

Conclusion: Taken together, these findings show that pharmacologic inhibition of elevated PAI-1 rescues the impairment in thrombus resolution observed in DM, and may have efficacy as a therapeutic strategy to prevent diabetic thromboembolism.

Disclosure of Interest: None declared.

Factor V and regulation

AS148

Factor V and TFPI interactions

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Coagulation Factor V (FV) circulates in plasma as an inactive prococfactor. Following B-domain removal, the active cofactor FVa, enhances the catalytic efficiency of FXa by several orders of magnitude. Previous findings have established that evolutionary conserved regions within the B-domain play a key role in keeping FV as an inactive prococfactor by, in part, concealing FXa binding site(s). These regions of the FV B-domain consist of basic (BR) and acidic (AR)

regions and define the minimal sequence necessary to maintain FV as a procofactor. Recent data have shown that removal of either one of these regions results in FVa-like activity and that B-domain fragments spanning the BR act in-trans to suppress the activity of FV variants bearing only the AR (J Biol Chem. 287: 26342–51, 2012, J Biol Chem. 288: 30151–60, 2013). Physiologically, forms of FV that are missing a BR but harbor an AR are released by activated platelets after a partial proteolysis and may be generated via FV activation by FXa. Interestingly, other forms of FV that only harbor an AR have been described. The variant FV-East Texas and FV-Amsterdam results in an alternatively spliced form of FV that has most of the B-domain removed but retains the AR (FV-short; J Clin. Invest. 123: 3777–87; 2013; Blood, 2015 In press). Together these forms of FV should be constitutively active. However, it is possible that physiological ligands that mimic the BR could inhibit their activity. Previous studies have identified tissue factor pathway inhibitor (TFPIa) as one of these potential ligands. Remarkably, the C-terminal segment of TFPIa shares substantial sequence homology with the FV BR and binds FV forms that only harbor the AR (PNAS, 110: 17838–43; 2013). While there are several remaining unanswered questions, the FV(a)-TFPIa interaction has the potential to fundamentally alter our understanding of cofactor regulation at the site of injury.

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AS149

FV enhances protein S cofactor function for TFPI in the inhibition of FXa

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Background: Protein S acts as cofactor for tissue factor pathway inhibitor (TFPI) through a direct interaction between the protein S SHBG-like domain and the TFPI Kunitz domain 3 that efficiently enhances factor (F) Xa inhibition. TFPI also binds FV and FVa in plasma and is capable of inhibiting prothrombinase through a direct interaction with FXa-activated FV and platelet-derived FV.

Aims: The role of the FV/TFPI interaction is not fully understood. We therefore evaluated the influence of FV upon TFPI inhibition of FXa in the presence and absence of protein S.

Methods: FXa inhibition assays with purified reagents were used to evaluate the enhancement of TFPI mediated inhibition of FXa by protein S and FV.

Results: Whereas FV alone did not influence TFPI-mediated FXa inhibition, it further enhanced TFPI function in the presence of protein S. Protein S primarily stimulated the formation of the initial FXa/TFPI complex, with a ~10-fold decrease in K_i. FV (and not FVa) further augmented the initial FXa/TFPI complex formation in a protein S dependent manner, causing a ~32-fold reduction of K_i compared to TFPI alone. FV together with protein S showed limited enhancement of the isomerization of FXa/TFPI to FXa/TFPI*. Anti-FV and anti-protein S antibodies demonstrated that the FV enhancement was specific and protein S-dependent.

TFPI with a truncated C-terminal tail (TFPI 1–249) was enhanced by protein S and FV, suggesting that the basic region in the TFPI C-terminal tail, previously shown to bind to the acidic region in FV, was not necessary for the FV cofactor function. Experiments using TFPI R199Q and TFPI E226Q, two variants with decreased ability to bind protein S, demonstrated that optimal interaction between TFPI and protein S was not required for FV cofactor function.

Conclusion: These results suggest that FV acts together with protein S as a synergistic cofactor for TFPI, a previously unknown anticoagulant function of FV.

Disclosure of Interest: None declared.

AS150

East Texas FV-Short/TFPI interaction in inhibition of FXa- and TF-initiated thrombin generation

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Background: In East Texas bleeding disorder a point mutation in exon 13 of FV gene results in alternative splicing and deletion of 702 residues of the B-domain generating FV-Short, which binds and retains TFPI in circulation resulting in a bleeding phenotype.

Aims: To elucidate whether FV-Short/TFPI inhibits both TF- and FXa-initiated thrombin generation (TG) and whether TFPI activity is decreased upon thrombin activation of FV-Short.

Methods: Combinations of intact or thrombin-activated recombinant FV-Short and TFPI were added to normal or FV-deficient plasma. After initiation of the reaction by either TF or FXa, TG was continuously monitored with fluorescent substrate.

Results: Using normal plasma (NP), TF-initiated TG was not affected by addition of intact or pre-activated rFV-Short and added TFPI dose-dependently inhibited TG, the results being similar whether rFV-Short was pre-activated or not. In contrast, in FXa-initiated reactions the addition of intact or pre-activated rFV-Short shortened the lag-phase. In this setting, addition of TFPI with intact rFV-Short resulted in stronger inhibition of TG than when pre-activated rFV-Short was used.

To avoid the influence of full-length FV in NP, FV-deficient plasma was reconstituted with rFV-Short ± TFPI. TFPI dose-dependently inhibited TG after initiation with either TF or FXa. The inhibition was stronger when intact rFV-Short was used as compared to pre-activated rFV-Short.

Conclusion: Our results suggest that in a plasma setting, intact rFV-Short was more efficient in supporting TFPI-mediated inhibition of FXa- and TF/FVIIa/FXa-induced thrombin generation than pre-activated rFV-Short. The results are also in agreement with the hypothesis that TFPI in complex with FV-Short is more efficient than free TFPI in inhibiting the TF/FVIIa/FXa-complex and free FXa.

Disclosure of Interest: None declared.

AS151

Development and characterization of an RNA aptamer targeting FV/FVa

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Background: Targeting of coagulation cascade cofactors, and cofactors in general, has remained a challenge due to their lack of an active site; however they represent potentially useful anticoagulant targets. We have developed an anticoagulant RNA aptamer that binds to FV and FVa that can be reversed by protamine sulfate.

Aims: In this study we aimed to generate an RNA aptamer with anticoagulant activity that bound to FV and FVa. Additionally, we characterized the anticoagulant activity with plasma-based assays and explored the mechanism of the aptamer.

Methods: SELEX to modified human FV was used to generate high affinity RNA aptamers. The lead aptamer was truncated and a mutant aptamer was created by altering 3 nucleotides to eliminate anticoagulant and binding activity. Approximate K_d values were measured using nitrocellulose filter binding assays. Anticoagulant activity was measured with a coagulometer using citrated, pooled normal plasma. Reversal of anticoagulant activity was measured via modified aPTT to include incubation with protamine sulfate. Thrombin generation in the presence of the RNA aptamer was quantified using a chromogenic thrombin substrate. The assays were performed with aptamer, modified FVa, FXa, phospholipids, and Q271 and des-gla-Q271 prothrombin.

Results: The aptamer can dose-dependently increase clotting time in aPTT and PT assays. Protamine sulfate is able to return clotting time to normal within 5 min of addition. The K_d to human FV is ~20 nM and to human FVa is ~1 nM. The aptamer can dose-dependently decrease thrombin generation as monitored by chromogenic substrate cleavage of thrombin. The mutant aptamer has no anticoagulant activity, does not bind to FV or FVa, and cannot reduce thrombin generation.

Conclusion: This aptamer exhibits anticoagulant activity in plasma-based assays through its ability to bind to FV and FVa. The likely mechanism of action is through the disruption of the FVa-prothrombin interaction based on biochemical assays.

Disclosure of Interest: E. Soule: None Declared, S. Krishnaswamy: None Declared, B. Sullenger Shareholder of: Regado Biosciences, Consultant for: Regado Biosciences.

Determinants of thrombus structure and susceptibility to lysis

AS152

Cellular and mechanical modulators of fibrin structure

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Fibrin stability and susceptibility to lysis is predetermined at the stage of clot formation when the relative kinetics of fibrinogen conversion to fibrin, the polymerization of fibrin monomers and the assembly of protofibrils in fibers define the final structure of the clot. Recently identified modifiers of fibrin structure improve our understanding of the role of plasmin as a major fibrinolytic protease. Platelets present at the site of fibrin formation modulate the structure chemically (through effects on thrombin concentration) and mechanically (through contractile force). Acceleration of the fibrinogen-fibrin conversion at higher concentrations of thrombin favours the longitudinal growth and branching of protofibrils over their lateral association with consequent resistance to plasmin. The contraction of fibrin-bound platelets and the mechanical shear generated by circulating blood profoundly alters the fibrin architecture generating fibers that are longitudinally aligned with smaller diameter and pore size. Factor XIIIa tightens the lateral attachment of protofibrils and decreases the volume of the vacant fluid space within the fibers, thus restricting the plasmin movement in the fibers. DNA and histones, components of the extracellular traps released by activated neutrophils in thrombi cause thickening of fibers through increase in the interprotofibril distances resulting in a both mechanically and enzymatically less vulnerable fibrin. Red blood cells (RBC) are involved in active interactions with fibrin through an integrin receptor. Thus, fibrin can transmit the contractile force of activated neighbouring platelets to RBC causing a change of their shape from biconcave to polyhedral with consequent almost perfectly gap-free compaction of RBC in the vacant space between fibers forming a stronger diffusion barrier to plasmin. These findings indicate that thrombus stability is determined by a complex interplay of mechanical, chemical and cellular mechanisms.

Disclosure of Interest: None declared.

AS153

Factor XIIIa crosslinking of fibrin α -chain mediates red blood cell retention in clots

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Background: Factor XIII(a) [FXIII(a)] stabilizes clots and increases resistance to fibrinolysis and mechanical disruption. Recently, we showed that FXIIIa also mediates red blood cell (RBC) retention in retracted clots and determines venous thrombus size (Aleman 2014, *JCI*). These data suggest FXIII(a) is a potential target for reducing venous thrombosis. However, the mechanism by which FXIIIa retains RBCs in clots is not known.

Aims: Determine the mechanism(s) mediating RBC retention in clots.

Methods: Human and murine clots were assessed by weight, RBC retention, and microscopy. FXIIIa substrate crosslinking to RBCs was assessed by flow cytometry and SDS-PAGE.

Results: In the presence of FXIIIa, RBC retention in clots was positively correlated with network density ($R^2 = 0.75$, $P < 0.00001$), indicating crosslinked fibrin sterically retains RBCs in clots. However, FXIIIa inhibition abrogated this correlation, revealing an independent contribution of FXIIIa to RBC retention. FXIIIa substrates could not be crosslinked to RBCs, suggesting FXIIIa does not crosslink RBCs directly to the clot. RBC retention was not reduced in clots from mice deficient in α_2 -antiplasmin, TAFI, or fibronectin, indicating RBC retention does not depend on these FXIIIa substrates. To determine the contribution of fibrin crosslinking to RBC retention, we reconstituted fibrinogen-deficient plasma with RBCs, platelets, and recombinant fibrinogen variants. FXIIIa inhibition reduced RBC retention in clots formed with fibrinogen that lacks γ -chain crosslinking sites (γ NNR, $P < 0.03$), but not in clots that lack α -chain crosslinking sites ($\alpha\alpha 251$, $P = 0.3$). FXIIIa inhibitor concentrations that primarily block α - but not γ -chain crosslinking decreased RBC retention in clots. These data indicate RBC retention in clots is mediated by α -chain crosslinking.

Conclusion: These data expose a newly-recognized, essential role for fibrin α -chain crosslinking during clot formation and consolidation, and establish FXIII as a key determinant of thrombus composition and size.

Disclosure of Interest: J. Byrnes: None Declared, C. Duval: None Declared, Y. Wang: None Declared, M. Mooberry: None Declared, S. Lord: None Declared, J. Meijers Employee of: Sanquin Research, H. Ni: None Declared, R. Ariens: None Declared, A. Wolberg: None declared.

AS154

Zn²⁺ accelerates clot formation, modifies clot structure, and promotes clot stabilityHenderson SJ¹, Xia J², Stafford AR³, Leslie BA³, Fredenburgh JC³, Weitz DA² and Weitz JI¹¹Biochemistry and Biomedical Sciences, Thrombosis and Atherosclerosis Research Institute, McMaster University, Hamilton, Canada; ²Engineering and Applied Sciences, Harvard University, Cambridge, Massachusetts, USA; ³Medicine, Thrombosis and Atherosclerosis Research Institute, McMaster University, Hamilton, Canada**Background:** Fibrin (Fn) clots are formed when thrombin converts fibrinogen (Fg) to fibrin (Fn) monomers, which polymerize and are cross-linked by factor (F) XIIIa. Zn²⁺ released from platelets increases the local concentration to 5–10 µM, and binds Fg and Fn and retards fibrinolysis. It is uncertain whether Zn²⁺ modulates Fg clotting and alters the structural or mechanical properties of Fn clots.**Aims:** To examine the effect of Zn²⁺ on clot formation and structure.**Methods:** Thrombin-induced clotting of Fg in buffer and plasma and polymerization of Fn monomers were monitored by absorbance. Fn structure was assessed using scanning electron microscopy (SEM), and the viscoelastic properties of Fn clots were determined by rheometry. Experiments were performed in the absence or presence of FXIIIa, and 5–10 µM Zn²⁺.**Results:** Zn²⁺ significantly ($P < 0.001$) accelerated (a) Fg clot time by 3.5- and 2.3-fold in the absence and presence of FXIIIa, respectively, (b) plasma clot time by 1.4-fold, and (c) Fn monomer polymerization by 2.5-fold. Absorbance in the presence of Zn²⁺ was up to 3.3-fold higher than in its absence, and SEM analysis revealed a significant ($P < 0.05$) 2-fold increase in Fn fiber diameter with Zn²⁺. FXIIIa increased the storage modulus of Fn clots from 128 ± 19 to 415 ± 27 Pa. However, Zn²⁺ reduced the storage modulus by 10- and 8.5-fold in the absence and presence of FXIIIa, respectively, and clots formed in the presence of Zn²⁺ exhibited a lower elastic modulus and resisted rupture. Therefore, Zn²⁺ accelerates Fg clotting and reduces Fn clot stiffness in a FXIIIa-independent manner.**Conclusion:** Although Zn²⁺ accelerates thrombin-mediated clotting of Fg, enhances Fn monomer polymerization, and increases Fn fiber thickness, it reduces clot stiffness both in the absence or presence of FXIIIa. Therefore, Zn²⁺ released from activated platelets at sites of vascular injury may accelerate clot formation and promote clot stability, thereby reducing the risk of embolization.**Disclosure of Interest:** None declared.

AS155

Thrombin and fibrinogen gamma' regulate protofibril packing within the fibrin fiber leading to changes in clot structure and strengthDomingues M^{1,2}, Duval C¹, McPherson HR¹, Ajjan RA¹, Ridger VC³, Connell SD², Philippou H¹ and Ariens RA¹¹Division of Cardiovascular and Diabetes Research, Faculty of Medicine and Health, University of Leeds; ²Molecular and Nanoscale Physics group, School of Physics and Astronomy, University of Leeds, Leeds; ³Department of Cardiovascular Science, Faculty of Medicine, Dentistry, and Health, University of Sheffield, Sheffield, UK**Background:** Blood coagulation culminates in the production of thrombin which converts fibrinogen into fibrin, forming the blood clot, in order to stop bleeding events. A common splice variant of fibrinogen, called fibrinogen γ' has been associated with several cardiovascular conditions. Previous studies have shown significant effects of thrombin and fibrinogen γ' on clot structure. However, much of the

structural information regarding fibrin clots was obtained using electron microscopy which requires sample dehydration and thereby affects fiber structure.

Aims: Our aim was to investigate the role of thrombin and fibrinogen γ' in the modulation of fibrin fiber structure, in purified systems, using clots under fully hydrated conditions.**Methods:** Fibrin fibers were studied using turbidimetry, atomic force microscopy and magnetic tweezers in purified systems.**Results:** In the range of concentrations used, thrombin induced a 4.5-fold decrease in average protofibril content per fibrin fiber, with a relatively minor decrease in fibrin fiber size of 1.5-fold, leading to the formation of a less compact fibrin fiber structure. Decreased protofibril content of the fibrin fibers relates to less stiff fibrin clot as analysed by a microrheometer based on magnetic tweezers. Fibrin fibers produced with fibrinogen γ' showed reduced protofibril packing at low thrombin concentrations.**Conclusion:** These findings demonstrate that in fully hydrated conditions, thrombin and fibrinogen γ' have more dramatic effects on protofibril content than fibrin fiber size and that protofibril density within fibrin fibers correlates with strength of the fibrin network. We conclude that regulation of protofibril content of fibrin fibers is an important mechanism by which thrombin and fibrinogen γ' modulate fibrin clot structure and strength.**Disclosure of Interest:** None declared.

AS156

The role of blood microparticles in the formation, structure, and properties of fibrin clotsLitvinov RI^{1,2}, Nabiullina RM³, Mustafin IG³, Weisel JW² and Zubairova LD³¹Kazan Federal University, Kazan, Russia; ²University of Pennsylvania, Philadelphia, USA; ³Kazan State Medical University, Kazan, Russia**Background:** Despite the importance of circulating microparticles (MPs) in hemostasis and thrombosis, little is known about their causative effects on clot formation and lysis, the final stages of blood clotting *in vivo*.**Aims:** To compare fibrin formation dynamics, clot structure, and susceptibility to fibrinolysis in the presence and absence of MPs.**Methods:** MPs were removed from platelet-free plasma (PFP) by filtration through a 0.1-µm-filter, yielding microparticle-depleted plasma (MDP). MDP was replenished with cephalin (MDP-C) to restore the phospholipids. Concentrations of MPs were assessed by flow cytometry. Fibrin polymerization and tPA-induced lysis were followed by dynamic turbidimetry. A thrombin generation test was performed using a chromogenic substrate. Fibrin ultrastructure was studied with scanning electron microscopy. Fresh samples of PFP, MDP, and MDP-C from the same healthy donors were analyzed in parallel.**Results:** In the presence of MPs (PFP), fibrin polymerization was accelerated and the final fibrin network structure was more compact and built of thinner fibers than in the absence of MPs (MDP). The less porous PFP-clots were significantly more resistant to both intrinsic and extrinsic lysis by plasmin than MDP-clots. Reconstitution of phospholipids (MDP-C) restored the fibrin polymerization kinetics, fibrin structure, and susceptibility to fibrinolysis, making them similar to PFP. The observed changes correlated with a decreased thrombin generation rate in MDP vs. PFP, which was fully restored after addition of exogenous phospholipids. In addition, the clots formed in PFP and MDP-C had 0.1–0.5-µm size granular material on fibers, suggesting that MPs and phospholipids can directly interact with fibrin.**Conclusion:** The blood of healthy individuals contains functional MPs that affect the structure and stability of fibrin clots indirectly through acceleration of thrombin generation and through direct physical incorporation into the fibrin network.**Disclosure of Interest:** None declared.

Prevention of thrombosis in children

AS157

Why do we need DVT prophylaxis in children?

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Venous thromboembolism (VTE) prophylaxis in children is emerging as an area for quality of care improvement. Multiple adult studies support the efficacy and safety of mechanical and pharmacological prophylaxis in patients at risk of VTE. There is an absence of similar data clearly defining the absolute benefit of VTE prophylaxis in children. The lack of established evidence-based protocols or randomized control trial data to drive protocol development is problematic. There are a number of reasons that VTE prophylaxis is relevant to current pediatric care including: the increasing rates of VTE over time in children, the potential short-term complications of VTE, and long-term impact of post-thrombotic syndrome, including impact on quality of life. Given the probable inappropriateness of adult regimes being applied to children without modification, there is a current need to develop a systematic approach to VTE prophylaxis in children. An individualized approach to VTE prophylaxis in patients can incorporate risk and benefit in decision making for a single patient. Risk stratification and predictive models for VTE are approaches that have been advocated. As the majority of VTEs in children are associated with frequently multiple underlying risk factors including central venous lines, sepsis, immobility, malignancy, surgery, congenital heart disease and trauma, further studies are needed to define the highest risk groups for VTE, to most appropriately implement prophylaxis regimes. Ultimately, appropriately targeted VTE prophylaxis in children is a necessary component of current pediatric care aimed at improving short and long-term outcomes.

Disclosure of Interest: None declared.

AS158

Blood group, age and type of cancer are independently associated with symptomatic venous thrombotic events in pediatric cancer patients: a population based study from maritimes, Canada

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Background: Symptomatic venous thrombotic events (sVTE) are a well-recognized complication in pediatric cancer patients (PCP). However, risk factors (RF) are unclear. Moreover, the limitations of several of the previously described RF for sVTE include (i) poor generalizability to all pediatric cancers, (ii) hemostatic protein lab values altered by the cancer itself, (iii) long turn-around times and (iv) testing restricted to specialized labs. There is a need to identify RF for sVTE in PCP that are easily ascertained at the time of cancer diagnosis.

Aims: Identify RF associated with sVTE in PCP: Methods: All PCP in the 3 Maritime Provinces of Nova Scotia, New Brunswick and Prince Edward Island are treated at IWK Health Center (IWK) in a shared care model, providing a population-based cohort. After ethics approval, all PCP treated at the IWK from 2000 to 2014 with sVTE were identified. Clinical and laboratory data was extracted from the: (i) Pediatric oncology hospital database (ii) Provincial Cancer in Young People registry (iii) Electronic medical records (iv) Pharmacy database and (v) Hospital health records. SPSS version 21 was used for statistical analysis.

Results: Forty-two ($4.8 \pm 0.07\%$) of the 875 patients had sVTE. The mean age at diagnosis for sVTE patients was 10.9 years. The gender ratio was M:F::0.9:1. Central veins (73.7%) were the most common location for sVTE. On univariate analysis, age > 10 years at diagnosis ($P = 0.001$), type of cancer ($P = 0.026$) and non-O blood group ($P = 0.007$) were associated with sVTE, while gender and type of chemotherapy were not. On multivariate analysis, age > 10 years (odds ratio [OR]: 2.9 [1.5–5.5], $P = 0.001$), non-O blood group (OR: 2.39 [1.15–4.98], $P = 0.020$) and type of cancer (brain tumor; OR: 0.1 [0.014–0.776], $P = 0.027$) were associated with sVTE. The area under the receiver operating characteristics curve for the model was 0.73.

Conclusion: In a large population based cohort of patients, we identified novel, easily available, and independent RF for sVTE in pediatric cancer patients.

Disclosure of Interest: None declared.

AS159

Venous thromboembolism occurring during adolescence: is it preventable?

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Background: Risk assessment for venous thromboembolism (VTE) and thromboprophylaxis (TP) in those with thrombotic risk factors is established in adult practice. Evidence to support efficacy and safety of this approach in adolescents is lacking.

Aims: We aimed to describe thrombotic risk factors and to determine the proportion of potentially preventable events in a cohort of adolescents with VTE.

Methods: Data were collected retrospectively over 6 years, 2008–2014, from 8 tertiary UK centres. Qualifying events were radiologically-confirmed VTE in subjects aged 12–17 years. Arterial and upper venous system line-related thrombosis was excluded.

Results: Seventy-six cases were identified, 41 males, median age 15 years. Site of thrombosis was: lower limb deep vein thrombosis (DVT), 43; cerebral venous sinus, 12; pulmonary embolism, 11; upper limb DVT, 5; other, 5. Six (8%) were line-related. Frequent risk factors were: reduced mobility, 34; thrombophilia, 21 (inherited, 17; acquired, 4), not known at presentation; malignancy, 15; surgery, 14; OCP, 9; congenital venous anomaly, 4. Median number of thrombotic risk factors was two. 28 presented as outpatients with no significant risk factor for VTE which was considered 'unpreventable'. Of 48 where there had been an opportunity for VTE risk assessment, i.e. were inpatients ($n = 28$) or outpatients with a known underlying diagnosis and/or provoking event within the prior 3 months ($n = 20$), 25 had no indication for TP and 9 had a contraindication. A further 5 who had received appropriate TP and 9 in whom TP was indicated with no contraindication but not given were considered 'potentially preventable' (14/76; 18%). Of these, 4 had cerebral palsy, 5 malignancy and 2 inflammatory bowel disease. All had reduced mobility with recent surgery in 8.

Conclusion: Amongst a cohort of adolescents with VTE, 18% were 'potentially preventable'. VTE risk assessment and preventative measures should be focused on those with significant immobility and surgery, particularly in the setting of malignancy.

Disclosure of Interest: None declared.

AS160

Venous thrombo-embolism (VTE) in children with sickle cell disease (SCD): an institutional experienceWoods G^{1,2}, Sharma R^{1,2}, Creary S^{1,2}, Dunn A^{1,2}, Hor K^{2,3}, Young J¹, Young C^{2,4} and Kumar R^{1,2}¹Division of Hematology/Oncology, Nationwide Children's Hospital; ²Department of Pediatrics, The Ohio State University; ³Division of Cardiology; ⁴Division of Radiology, Nationwide Children's Hospital, Columbus, USA**Background:** Patients with SCD manifest systemic coagulation activation thought to result in a chronic hypercoagulable state. Adult studies have indicated an increased incidence of VTE in SCD.**Aims:** To evaluate the prevalence, risk factors and clinical course of VTE in children with SCD at Nationwide Children's Hospital (NCH).**Methods:** Permission for this retrospective study was obtained from the IRB. All pediatric patients (2–21 years) with SCD (SS, SC, Sp⁺ and Sp⁰ genotypes) followed at NCH from 2009 to 2015 were evaluated. VTE was defined as positive imaging (ultrasound, CT angiography, magnetic resonance imaging) in a symptomatic patient. Demographic data was collected from the electronic medical records. Standard statistical methods were used to summarize findings (mean $\pm$ SD). Fisher's exact test was used to assess significance.**Results:** A total of 365 patients (186 males, 179 females) patients with SCD (200 SS, 123 SC, 32 Sp⁺ and 10 Sp⁰) were followed at NCH during the study period (2190 patient years). VTE was documented in 12 patients (4 males, 8 females), 9 of which were central venous line (CVL) associated. 1 patient had isolated PE (patient had a CVL, but line was not imaged), 1 had leg vein thrombosis and 1 had sinus venous thrombosis. Mean age at VTE diagnosis was 15.5 ($\pm$ 3.7) years. On univariate analysis, SS genotype ($P = 0.014$) and presence of CVL ($P < 0.001$) were associated with VTE. Thrombophilia testing was completed on 10 patients-6 had low levels of protein C and/or protein S and 1 was heterozygous for FV Leiden. All patients were treated with anticoagulation and 2 patients underwent thrombolysis. Mean duration of anticoagulation (therapeutic and prophylactic) was 16.4 ($\pm$ 15) months. 3 patients with CVL had clot progression/recurrence on anticoagulation therapy. CVL has since been removed on 8/10 patients.**Conclusion:** Prevalence of VTE in children with SCD is high. Sickle cell genotype and CVL are associated with VTE. We are currently re-evaluating our institutional policy for CVL use in children with SCD.**Disclosure of Interest:** None declared.

AS161

Hospital-associated venous thromboembolism (HA-VTE) IN pediatrics: a systematic review and meta-analysis of risk factors and risk assessment modelsMahajerin A^{1,2}, Branchford BR³, Amankwah EA⁴, Raffini L⁵, Chalmers E⁶, van Ommen CH⁷ and Goldenberg NA⁸¹Pediatrics, University of California, Irvine; ²Hematology, CHOC Children's Specialists, Orange; ³Pediatrics, University of Colorado, Denver, Denver; ⁴Clinical and Translational Research, All Children's Research Institute, St. Petersburg; ⁵Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, USA; ⁶Haematology, Royal Hospital for Sick Children, Yorkhill, Glasgow, UK; ⁷Pediatric Haematology, Emma Children's Hospital, Amsterdam, The Netherlands; ⁸Pediatrics, John Hopkins University School of Medicine, St. Petersburg, USA**Background:** Hospital-associated venous thromboembolism (HA-VTE) is increasing in pediatric centers. There is a need for guidelines on safe, effective, and evidence-based prevention but the lack of high-quality evidence has impeded development of such guidelines.**Aims:** The objective was to systematically review literature on pediatric HA-VTE risk factors and risk-assessment models, to inform future pediatric HA-VTE prevention research.**Methods:** We conducted a literature search on pediatric VTE risk via PubMed (1946–2014) and Embase (1980–2014). Data on risk factors and risk-assessment models were extracted from case-control studies, while prevalence data on clinical characteristics were obtained from VTE registries, large ($n > 40$) retrospective case series, and cohort studies. Meta-analyses were conducted for risk factors or clinical characteristics reported in at least 3 studies. Heterogeneity among studies was assessed with Cochran's Q and quantified by I² statistic.**Results:** From 394 initial articles, 60 met final inclusion criteria (19 case-control studies and 41 registries/large case series/cohort studies). Significant HA-VTE risk factors among case-control studies were: systemic infection (OR: 2.42, 95% CI 1.52–3.85); intensive care unit (ICU) stay (OR: 2.14, 95% CI 1.97–2.32); central venous catheter (CVC; OR: 2.13, 95% CI 2.00–2.27); mechanical ventilation (OR: 1.56, 95% CI 1.42–1.72); and hospital length of stay (LOS) (per each additional day, OR: 1.03, 95% CI 1.03–1.03). 3 studies developed/applied risk-assessment models from a combination of these risk factors. Fourteen significant clinical characteristics were identified through non-case-control studies.**Conclusion:** This meta-analysis confirms systemic infection, CVC, ICU stay, mechanical ventilation, and LOS as pediatric HA-VTE risk factors. A few pediatric HA-VTE risk scores have emerged employing these factors. Prospective validation is necessary to inform risk-stratified pediatric HA-VTE prevention trials.**Disclosure of Interest:** None declared.

Pregnancy related bleeding complications

AS162

Management of reproductive hemorrhagic complications

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This presentation will review the obstetric, gynecologic and hemostatic management of reproductive hemorrhagic complications and will briefly review pertinent reproductive physiology. Most reproductive hemorrhagic complications are uterine in origin. In nonpregnant women, uterine bleeding is most often due to ovulatory dysfunction or abnormalities of the uterine cavity. Reproductive hemorrhagic complications are more likely to occur in women with underlying bleeding disorders. Conversely, about 10–20% of women with heavy menstrual bleeding have been found to have underlying bleeding disorders. Reproductive hemorrhagic complications in nonpregnant women can be effectively managed by hormonal, surgical and hemostatic therapies. The first step in the evaluation of a patient is to determine whether she is pregnant or not. In the first half of pregnancy, most hemorrhagic complications are due to miscarriage or ectopic pregnancy. In the second half, most are due to preterm labor or abnormal placentation. At the time of delivery, approximately 80% of hemorrhagic complications are obstetrical, 20% are surgical and a small percentage are hemostatic. Inherited bleeding disorders increase the risk of obstetrical hemorrhage 2 to 3-fold but are rarely the cause of massive hemorrhage. Massive hemorrhage, which occurs in 0.15% to 0.5% of deliveries, is usually due to an acute acquired coagulopathy most often caused by profuse obstetrical or surgical bleeding. Management of obstetrical bleeding requires uterine evacuation and uterotonics; and sometimes balloon tamponade, compression sutures or hysterectomy. Management of surgical bleeding requires appropriate surgical repair. Management of coagulopathy requires early recogni-

tion, laboratory monitoring, correction of metabolic defects, blood products, replacement of clotting factors, antifibrinolytics and the combined expertise of obstetricians, anesthesiologists, transfusion medicine experts and hematologists.

Disclosure of Interest: None declared.

AS163

Transfusion protocol in severe postpartum hemorrhage

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Background: The use of pharmacological agents and new surgical techniques to control severe postpartum hemorrhage (sPPH) have failed to reduce the incidence of emergency postpartum hysterectomy.

Aims: To evaluate clinical outcome and effect of a standard transfusion protocol including early administration of hemostatic agents and recombinant activated factor VII (rFVIIa) in sPPH after vaginal or caesarean delivery.

Methods: Single-center observational cohort study performed at our tertiary care university hospital. Standard treatment protocol for sPPH included early administration of uterotonic drugs, crystalloids, tranexamic acid, blood products, fibrinogen and rFVIIa. Main outcome measures were bleeding volume, transfused products and hysterectomy. The cohort was compared to patients treated before the introduction of this protocol.

Results: During the 8-year study period 74 patients with sPPH were treated with three different therapeutic strategies: 'A' An in-house protocol regulating the use of rFVIIa (36 months; $n = 20$); 'B' Absence of an in-house guideline (27 months, $n = 27$), and 'C' The above mentioned standardized treatment algorithm (33 months; $n = 27$). Treatment with the standard transfusion protocol 'C' was associated with a significant reduction of bleeding volume compared to strategies 'A' and 'B' ('C' median 3000 mL [IQR: 2000–4000 mL], 'A' 4500 mL [IQR: 3000–5500 mL] and 'B' 6000 mL [IQR: 3000–6500 mL], $P = 0.004$), transfused erythrocyte concentrates ('C' $n = 6$ [IQR: 3–9], 'A' $n = 12$ [IQR: 8–16] and 'B' $n = 12$ [IQR: 6–16], $P = 0.007$), fresh frozen plasma ('C' $n = 5$ [IQR: 2–8], 'A' $n = 10$ [IQR: 7–13] and 'B' $n = 10$ [IQR: 4–12], $P = 0.004$) and emergency postpartum hysterectomy ('C' $n = 1$ [3.7%], 'A' $n = 5$ [25%] and 'B' $n = 10$ [37%], $P = 0.012$).

Conclusion: Implementation of standardized institutional approach to the management of sPPH is recommended. Transfusion protocols should consider early administration of tranexamic acid and hemostatic products, including rFVIIa, in addition to uterotonic drugs and blood products.

Disclosure of Interest: None declared.

AS164

Management of 40 deliveries in 28 women with type 1 and type 2 von Willebrand disease

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Background: Post-partum hemorrhage (PPH) is reported in up to 37% of deliveries among women with Von Willebrand disease (VWD). While women with type 3 VWD undoubtedly require replacement therapy, recommendations for women with type 1 and 2 are still debated.

Aims: We report our experience in the delivery of women with VWD in light of published guidelines.

Methods: We followed 40 consecutive deliveries (28 vaginal deliveries (VD) and 12 cesarean sections (CS)), between 1992 and 2014, in 28 women with VWD (9 type 1, 19 type 2 excluding type 2B). Eight women had basal ristocetin cofactor activity (VWF:RCo) < 10 UI dL⁻¹, 15 women had VWF:RCo between 10 and 30 UI dL⁻¹ and the 5 women with type 2N VWD had basal factor VIII (FVIII:C) ≤ 30 UI dL⁻¹.

Results: At the end of pregnancy, VWF:RCo was < 50 UI dL⁻¹ in 18 women (25 deliveries) with FVIII:C < 50 UI dL⁻¹ in 3 women (4 deliveries); FVIII:C was < 50 UI dL⁻¹ in 3 women (4 deliveries) with type 2N. Neuroaxial anesthesia was performed for 7 deliveries in women with factors levels ≥ 49 UI dL⁻¹. Desmopressin has been infused for 19 deliveries (12 VD and 7 CS): one single infusion in 14 cases, a second one 12 h later in 5 cases and a third one 36 h later in 1 case. One PPH (2.5%), attributed to uterine atony after a CS with desmopressin therapy, was solved by sulprostone and tranexamic acid. Six excessive bleeding were treated by desmopressin ($n = 6$) and manual exploration of the uterine cavity ($n = 4$). Profuse lochia in 2 women, hemorrhagic resumption of menses (without loss of hemoglobin) in 1, and wound hematoma in 1 required oral tranexamic acid. Eleven deliveries in 9 women with VWF:RCo < 50 UI dL⁻¹ and/or FVIII:C < 50 UI dL⁻¹ occurred without bleeding complication in the absence of treatment. No women received Von Willebrand factor concentrate or blood transfusion.

Conclusion: Our experience suggests that replacement therapy should not be systematic in women with type 1 or 2 VWD (except type 2B), even in the absence of correction of VWF:RCo and/or FVIII levels at the time of delivery.

Disclosure of Interest: None declared.

AS165

Postpartum bleeding and neuroaxial anesthesia in patients with factor XI deficiency

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Background: Factor XI deficiency (fXIId) is a rare bleeding disorder. Affected patients are at increased risk of bleeding, yet fXI activity levels (fXIa) do not reliably predict bleeding complications. Variable bleeding rates make peripartum management of women with fXIId a challenge.

Aims: To characterize rates of peripartum bleeding and complications of neuroaxial anesthesia in patients with fXIId

Methods: After approval from the institutional review board, we reviewed the charts of all female patients with fXIId seen by our hematology practice between 2010 and 2014. We reviewed fXIa, obstetric and bleeding history, and peripartum complications. Severity of disease was characterized by fXIa $> 15\%$ (partial deficiency (PD)) or $\leq 15\%$ (severe deficiency – SD).

Results: Our analysis included 13 patients, 36 pregnancies and 34 term deliveries. Median baseline fXIa was 38% (1–58%). Median baseline aPTT was 35.4 s (28.1–53.2 s).

Thirty-four pregnancies were carried to term–26 (76.5%) in patients with PD, 8 (23.5%) in patients with SD and 25 (73.5%) in patients with bleeding history. Subchorionic hematoma occurred in 2 pregnancies (6.25%), both with PD and bleeding history. Two deliveries were complicated by post-partum hemorrhage (1 SD, 1 PD); the patient with SD had received prophylactic FFP and both patients had bleeding histories (menorrhagia, gingival bleeding). Overall 32 (94.1%) deliveries were uncomplicated, despite the use of prophylaxis in only 2 (6.35%) deliveries.

Neuroaxial anesthesia was used in 12 deliveries; all cases were in patients with PD, and 7 (58.3%) were in patients with bleeding histories. Prophylactic factor was not used in any case, and no patient experienced complications.

Conclusion: In our small retrospective analysis delivery was uncomplicated in the majority of patients with FXd, including patients with SD. There were no complications of neuroaxial anesthesia in patients with PD. Prospective studies are needed to further characterize which patients require prophylactic treatment.

Disclosure of Interest: None declared.

AS166

Endothelium drives a pre-dic state in endotoxemia with enhanced thrombosis and prolonged bleeding secondary to impaired TIE2 function

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Background: DIC is characterized by a consumptive coagulopathy that results in both microvasculature occlusion and uncontrolled bleeding. However, the acute effects of endotoxin that occur prior to dysregulation of the coagulation cascade are not well-studied.

Aims: Our aim was to identify endothelial responses to endotoxemia that initiate DIC.

Methods: We monitored laser-induced thrombus formation in cremaster arterioles 1–3 h following IP injection of LPS. Bleeding times were also evaluated.

Results: Platelet accumulation at injury sites following LPS exposure was increased to 198% of control values ($P = 0.02$). Tail clip assays demonstrated prolonged bleeding times in mice exposed to LPS for 3 h ($P < 0.05$). To determine whether the abnormalities in platelet accumulation and bleeding were secondary to coagulation defects at these early time points, we evaluated PT, aPTT, and platelet counts in LPS-treated mice, which were all similar to untreated controls. We also found no differences in PAR4-mediated platelet aggregation in LPS-treated mice. These observations lead us to evaluate whether endothelial abnormalities contribute to the early LPS-induced bleeding tendency. Previous studies have shown that endotoxemia results in substantially decreased function of the endothelial receptor Tie2. We therefore evaluated thrombosis in Tie2^{+/-} mice. There was a non-significant trend towards increased platelet accumulation and a significant increase in fibrin formation in Tie2^{+/-} mice compared with WT controls. To evaluate whether activation of Tie2 could reverse the LPS-induced prolongation of bleeding times, WT mice were injected with an angiopoietin-1 (a Tie2 receptor agonist) adenovirus vector prior to LPS exposure. Injection of angiopoietin-1 adenovirus protected mice from LPS-induced prolongation of bleeding time.

Conclusion: These studies demonstrate that the bleeding propensity in DIC begins prior to the consumptive coagulopathy and may result from impaired Tie2 signaling.

Disclosure of Interest: None declared.

Platelet structure and function

AS167

The role of gap junctions in platelet function

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Platelet activation at sites of arterial injury culminates in the assembly of a thrombus, a process that is triggered by an array of pro-thrombotic factors. Extensive data supports the notion that platelet cell signalling does not cease with the formation of a thrombus, but instead sustained contact-dependent signalling persists within the thrombus, that is import for its stability, contraction, and potentially resolution. Reports of the abilities of leukocytes to form gap junctions, led us to test the hypothesis that platelets possess gap junction (GJ) proteins (connexins) that may support direct intercellular signalling between platelets to enable coordinated control of thrombus formation and function. Platelets were found to possess several connexin family members, with notable levels of Cx37 and Cx40, and electron microscopy analysis of thrombi revealed structures with the typical appearance of GJs. Intercellular communication through GJs was confirmed using fluorescence recovery after photobleaching which was prevented by selective connexin inhibitors. Inhibition or deletion of connexins in platelets was found to prevent clot retraction, a process that we envisage requires coordination between platelets. Prior to docking between cells to form GJs connexins have been shown to display 'hemichannel' function. Single cell analysis of platelets revealed a role for Cx37 and Cx40 hemichannels in the control of platelet activation. The inhibition or deletion of connexins on platelets revealed a fundamental role for hemichannels and GJs in haemostasis and thrombosis, raising the possibility that their regulation may provide a new anti-thrombotic avenue. Towards this and through the use of micro-arrayed adhesive agonists and high speed live cell imaging, we have developed a platform to determine the mechanisms through which connexin function may be regulated and to establish the nature of molecules conveyed through these structures in platelets.

Disclosure of Interest: None declared.

AS168

Integrin PSI domain has endogenous thiol isomerase function and is a novel target for anti-thrombotic therapy

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Background: Integrins are a large family receptors expressed on almost all cells. Integrin β subunits contain a highly conserved PSI domain.

The CXXC motif, the active site of protein disulfide isomerase (PDI), is expressed twice in this domain of all integrins across species. However, the role of PSI domain in integrins and whether it contains thiol isomerase activity have not been explored.

Aims: To investigate the thiol isomerase activity of the PSI domain of integrins and its effect on $\beta 3$ integrin activation.

Methods: We generated recombinant PSI (rPSI) domains of murine $\beta 3$, human $\beta 1/\beta 2$ integrins, $\beta 3$ rPSI mutants, and detected their endogenous PDI-like activity by refolding of denatured RNase. We also developed mouse anti-mouse $\beta 3$ PSI domain monoclonal antibodies (mAb) in $\beta 3^{-/-}$ mice and detected their anti-thrombotic effect using two complimentary intravital microscopy models and a carotid artery thrombosis model.

Results: We identified that all integrin PSI domains tested have endogenous PDI-like activity, and both CXXC motifs of $\beta 3$ integrin PSI domain are required to maintain its optimal enzyme function since mutating one or both of them decreased or abolished their PDI-like activity. Through a competitive binding assay and western blot analysis, we demonstrated our anti-PSI mAbs have different binding affinities to rPSI and their mutants, suggesting diversity among them. These mAbs cross-reacted with $\beta 3$ PSI domains of human and other species and specifically inhibited their PDI-like activity. Similar to bacitracin (a PDI inhibitor), these mAbs inhibited integrin activation and reduced PAC-1 binding to purified human $\beta 3$ integrin. Importantly, these mAbs abrogated murine and human platelet aggregation *in vitro*, thrombus growth *ex vivo* and *in vivo* in both small and large vessels.

Conclusion: The PSI domain has endogenous PDI activity and is a key regulator of integrin activation. These findings should have broad implications for all integrin functions and provide a novel target for anti-thrombotic therapies.

Disclosure of Interest: None declared.

AS169

The wave complex member Hem1 is a critical regulator of platelet size and function in mice

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Background: Regulation of the actin cytoskeleton is essential for platelet production by megakaryocytes (MK) and for proper platelet function. The haematopoietic cell-specific adaptor protein Hem1 is a component of the WAVE complex which is activated by the Rho GTPase Rac1 and promotes actin polymerisation. Although the Rac1 effectors WAVE, PAK and Arp2/3 are supposed to transmit diverse signalling events, their precise roles in regulating platelet biogenesis and function have remained elusive.

Aims: We tested the hypothesis that Hem1 is an important regulator of platelet activation and cytoskeletal rearrangements.

Methods: Platelet function was assessed in mice with a constitutive deficiency of Hem1 using flow cytometric analysis of cellular activation processes and flow adhesion systems. GPIIb/IIIa-mediated outside-in signalling was analysed by performing platelet spreading assays. Platelet and MK morphology were assessed *in vitro* and *in situ* by confocal and transmission electron microscopy. *In vivo* function of platelets was studied in a model of arterial thrombosis.

Results: Ablation of Hem1 resulted in a dramatic increase in platelet size without affecting platelet counts. The number of bone marrow MKs were slightly reduced and they exhibited (i) a thickened peripheral zone, (ii) large platelet territories, or (iii) severely altered morphology. Hem1-deficient platelets showed impaired degranulation and markedly reduced integrin activation upon stimulation of GPVI, CLEC-2 or G protein-coupled receptors, resulting in impaired adhesion and throm-

bus formation on collagen under flow. Furthermore, spreading on fibrinogen was impaired in Hem1-deficient platelets, whereas F-actin content and polymerisation were unaltered. *In vivo*, these defects translated into a protection from arterial thrombus formation.

Conclusion: Our results reveal a crucial role of Hem1 in platelet production and function, while it is dispensable for general actin assembly in these cells.

Disclosure of Interest: None declared.

AS170

VAMP-7 links granule exocytosis to platelet spreading

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Background: Physiologic platelet granule secretion occurs following adhesion of platelets to injured vasculature. However, granule exocytosis has historically been studied in suspension platelets, while the mechanisms of granule exocytosis in adherent platelets remains poorly understood. VAMP-7 is a unique SNARE that contains a N-terminal profilin-like domain capable of associating with actin binding proteins and, thus, may serve as a link between platelet granule secretion and spreading.

Aims: To evaluate the role of VAMP-7 in platelet granule release and spreading.

Methods: We studied platelets from VAMP-7^{-/-} mice in assays of exocytosis and spreading.

Results: VAMP-7^{-/-} platelets demonstrated defective agonist-induced dense granule exocytosis and impaired aggregation compared to WT controls. α -Granule exocytosis from VAMP-7^{-/-} platelets was diminished both *in vitro* and *in vivo* during thrombus formation. Exocytosis defects were overcome at higher agonist concentrations, indicating normal cargo stores. Immunoprecipitation of VAMP-7 from platelet lysates followed by mass spectroscopy indicated interactions of VAMP-7 with the actin-binding complex Arp2/3, VARP, and filamin A. Results were confirmed by immunoblot analysis. To determine whether platelet spreading contributes to granule release, P-selectin expression in the presence of the small molecule Arp2/3 complex inhibitor, CK869, was evaluated. CK869 blocked granule release as indicated by P-selectin expression in spreading platelets, but not platelets in suspension, demonstrating that actin polymerization during spreading contributes to platelet granule release. Conversely, spreading of VAMP-7^{-/-} platelets on collagen was decreased by > 50% ($P < 0.05$) compared to WT controls, demonstrating that granule secretion contributes to platelet spreading.

Conclusion: VAMP-7 links granule exocytosis with actin polymerization and its interaction with cytoskeleton is crucial for normal spreading and granular release in platelets.

Disclosure of Interest: None declared.

AS171

Shear-induced GPIb-IX signaling via unfolding of its juxtamembrane mechanosensory domain

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Background: Platelets sense shear forces while responding to vascular injury through the interaction of GPIb-IX to VWF. How GPIb-IX

functions as a shear sensor and receptor is not clear. We recently identified a mechanosensitive domain (MSD) in GPIIb/IIIa that unfolds upon mechanical pulling on the engaged A1 domain of VWF. The unfolding force of MSD is relatively small, suggesting that physiological shear may trigger GPIIb/IIIa signaling.

Aims: To demonstrate that physiological shear induces deformation of MSD and transmits signals into the platelet.

Methods: Uniform shear stress ($0\text{--}30\text{ dyn cm}^{-2}$) was applied to fresh citrated human PRP via a cone-plate viscometer. Botrocetin (Bt) was added to induce VWF binding. Unfolding of MSD in GPIIb/IIIa and platelet signaling were monitored by FACS using site-specific fluorescent probes. Also the filopodia extension of stable CHO cells expressing GPIIb/IIIa, consequent upon adhesion on the VWF/Bt-coated surface and indicative of GPIIb/IIIa signaling, was analyzed by confocal microscopy.

Results: Shear induced Bt-dependent crosslinking of platelets and markedly enhanced binding of antibody 5G6 to its 10-residue epitope in the MSD, but not that of antibody RAM.1 to GPIIb/IIIa, on these platelets. 5G6 and RAM.1 do not interfere with each other's binding to GPIIb/IIIa. Bt+shear, but not Bt alone, induced a drastic change in FRET between donor-5G6 and quencher-RAM.1, and concurrently increased P-selectin expression in platelets. Partial deletions of MSD, which disrupted the integrity of MSD, significantly enhanced the filopodia extension in CHO cells expressing mutant GPIIb/IIIa, providing further support for a critical role of MSD in mediating GPIIb/IIIa signaling.

Conclusion: Upon VWF binding and physiological shear, the MSD in GPIIb/IIIa is unraveled and transmits signals into the platelet. These results demonstrate that MSD in GPIIb/IIIa senses and responds to physiological shear, providing critical insights on the pathophysiology of von Willebrand disease.

Disclosure of Interest: None declared.

Vascular remodeling

AS172

Molecular parallels between neural and vascular development

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Anatomical parallels between the nervous and the vascular system are readily apparent in peripheral body tissues, where blood vessels and nerves ramify throughout nearly all domains of the body and are usually aligned. To orchestrate the formation of their highly branched, exquisitely wired networks, nerves and blood vessels have developed shared cellular and molecular principles. At the cellular level, axons of developing neurons and capillaries use specialized motile structures to ensure their directional guidance. In axons, a growth cone is situated at the axon extremity and ensures axon guidance towards its distant target. In blood vessels, specialized motile endothelial cells (EC) situated at the capillary tips ensure capillary guidance during sprouting angiogenesis. Molecularly, common signaling molecules guide vascular and axonal outgrowth. Axonal growth cones and tip cells express receptors for axon guidance molecules, including Neuropilin receptors (Nrp), Eph family receptor tyrosine kinases, PlexinD1, Robo4 and UNC5B. Loss-of-function of the genes encoding these receptors leads to defects in vessel formation and in most cases to embryonic death, indicating a critical function of axon guidance receptors in vascular development. Their guidance properties and vascular expression makes them attractive targets for approaches directed at inhibiting tumor angiogenesis, or conversely for guiding new vessels towards ischemic tissue areas.

Disclosure of Interest: None declared.

AS173

Extracellular histones exert a differential cytotoxic and antiangiogenic effect in progenitor and mature endothelial cells

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Background: Histones are main components of neutrophil extracellular traps, which are highly augmented not only in infection but also in sites of neovessel formation, like peritumoral and regeneration tissues. Extracellular histone cytotoxic effect on endothelial cells has been previously studied, although the mechanism involved are not fully understood.

Aims: To further analyze histone cytotoxicity in endothelial cells and whether histones affect endothelial angiogenic functions.

Methods: Endothelial cells from micro (HMEC1) and macrovascular (HUVEC) beds and late outgrowth endothelial colony forming cells (ECFC) were treated with individual human recombinant histones. $P < 0.05$, ANOVA, $n = 5\text{--}8$.

Results: All histones triggered both necrosis and apoptosis in all cell types (fluorescence microscopy). Apoptosis was fully suppressed by the pancaspase inhibitor Z-VAD-fmk (98–100%) while necrosis was partially reduced (65–75%). Flow cytometric analysis of Ki67/PI staining showed that H2B, H3 and H4 induced cell cycle arrest of ECFC (17 ± 3 , 21 ± 4 , $22 \pm 3\%$ of cell in G0), HUVEC (31 ± 2 , 29 ± 5 , $59 \pm 6\%$) and HMEC1 (25 ± 4 , 31 ± 2 , $22 \pm 4\%$) and all histones exert a similar inhibition of EGM2- and SDF1-driven transwell migration of ECFC (58 ± 6 and $74 \pm 3\%$), HUVEC (55 ± 5 and $45 \pm 4\%$) and HMEC1 (25 ± 2 and $30 \pm 3\%$). Tubule formation on matrigel was also selectively impaired by histones. This inhibitory effect correlated with p38 phosphorylation levels (immunoblot) and was significantly prevented by the p38 inhibitor SB203580 (75–90%). Histone-mediated cytotoxic and antiangiogenic effects were fully suppressed by unfractionated and low molecular weight heparins (95–100%) and reduced by TLR2 and TLR4 blocking antibodies (55% and 65%).

Conclusion: Our results show that histones exert a differential cytotoxic and antiangiogenic effect not only in mature endothelial cells but also in late outgrowth endothelial progenitor cells, and point out heparin and TLR2/TLR4 blockade as possible therapeutic strategies to improve tissue regeneration.

Disclosure of Interest: None declared.

AS174

Blood cells-derived matrix metalloproteinase (MMP)-2 contributes to abdominal aortic aneurysm (AAA) development in a mouse model of hypertension/hypercholesterolemia

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Background: MMPs, and in particular MMP-2 and MMP-9, are involved in AAA formation by degrading extracellular matrix components. Platelets and circulating leukocytes contain and release MMP-2 but their role in AAA formation is not known.

Aims: Aim of our study was to evaluate the contribution of blood cell-derived MMP-2 to AAA formation in a mouse model of hypertension/hypercholesterolemia.

Methods: We generated a new mouse strain, LDLR/eNOS^{-/-} mice (dKO), in which hypertension and hyperlipidemia are simultaneously present. We then developed chimeric mice by cross-transplanting bone marrow from MMP-2^{-/-} mice into the dKO mice. After 16 weeks of

high fat diet blood pressure, cholesterol level, AAA formation and expression of MMP-2 in abdominal aortic extracts were evaluated.

Results: Blood pressure was increased equally in dKO and in eNOS^{-/-} mice (139 ± 4.2 and 136 ± 2.8 vs. 102 ± 6 mmHg in LDLR^{-/-}); total serum cholesterol was enhanced equally in dKO and in LDLR^{-/-} mice (25.3 ± 0.8 and 24.4 ± 0.3 vs. 4.98 ± 0.34 mmol L⁻¹ in eNOS^{-/-}).

Ten of 18 (~55%) of dKO mice developed AAA vs. none of LDLR^{-/-} (0/18, 0%) and of eNOS^{-/-} mice (0/8, 0%; $P < 0.0001$). dKO mice transplanted with bone marrow from MMP-2^{-/-} mice did not develop AAA (0/7, 0%). MMP-2 in AAA extracts of dKO animals was significantly enhanced as compared to LDLR^{-/-} (0.46 ± 0.11 vs. 0.18 ± 0.1 ng μg^{-1} of proteins) while it was unchanged in chimeric mice (0.15 ± 0.08 ng μg^{-1} of proteins). Moreover, circulating platelet P-selectin expression ($52 \pm 4\%$ vs. $71 \pm 5\%$, $P < 0.05$) and platelet leukocyte complexes ($17 \pm 6\%$ vs. $37 \pm 3.7\%$, $P < 0.05$) were reduced in chimeric mice as compared to dKO mice.

Conclusion: Our data show that hypertension together with hypercholesterolemia induce the generation of AAA by facilitating the penetration of blood-borne inflammatory cells expressing MMP-2 in the abdominal aorta. This new murine model can be useful for the assessment of new therapies aimed at reducing AAA development.

Disclosure of Interest: None declared.

AS175

Mechanism of regulation of VEGF-A by junctional adhesion molecule-A, an endogenous suppressor of angiogenesis

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Background: Vascular endothelial growth factor-A (VEGF-A) expression is normally suppressed in quiescent endothelial cells, but is upregulated during ischemia and tumor growth.

Aims: To delineate the mechanism of suppression of VEGF-A expression by junctional adhesion molecule-A (JAM-A), a tight junction protein.

Methods: *In vivo* and *ex vivo* angiogenesis assays and tumor growth were performed using *Jam-A* null mice with wild-type (WT) mice used as control. Vascular permeability was assessed by Miles assay. Q-PCR and Western blot analyses were used to assess gene expression.

Results: Congenic *Jam-A* null mice showed significantly enhanced ($P < 0.004$) vascular permeability. Plasma levels of VEGF-A in *Jam-A* null mice were age-dependently increased ($P < 0.001$) compared to WT mice. mRNA levels of VEGF-A were increased ($P < 0.001$) in *Jam-A* null endothelial cells (EC). We found Hypoxia inducible factor-1 α (HIF1- α) mRNA expression was amplified ($P < 0.02$) in EC lacking *Jam-A*. Interestingly, VEGF-A-induced angiogenesis was enhanced ($P < 0.001$) in the absence of *Jam-A*. Vascular EC isolated from *Jam-A* null mouse aorta showed enhanced ($P < 0.05$) cell migration and tube-like structure formation in response to VEGF-A. Furthermore, both mRNA and protein levels of VEGFR2, but not soluble Flt (sFlt), which is known to trap VEGF-A, were also increased ($P < 0.001$) in *Jam-A* null EC. The mRNA and protein expression of inhibitor of DNA binding 1 (Id1), a transcription factor known to upregulate VEGFR2 gene, were augmented ($P < 0.02$) in EC lacking *Jam-A*. Consistent with this finding, the overexpression of JAM-A in HUVECs attenuated the levels of Id1. Enhanced tumor growth and angiogenesis as well as vascular permeability in *Jam-A* null mice were completely inhibited by blocking VEGFR2 using anti-VEGFR2.

Conclusion: JAM-A suppresses VEGF-A/VEGFR2 expression in quiescent EC by attenuating transcription factors HIF-1 α and Id1 expression.

Disclosure of Interest: None declared.

AS176

Platelet endothelial aggregation receptor-1: a novel modifier of neoangiogenesis

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Background: Platelet Endothelial Aggregation Receptor-1 (PEAR1) is a cell membrane protein, expressed on platelets and endothelial cells (ECs). PEAR1 sustains $\alpha\text{IIb}\beta 3$ -activation in aggregating platelets and attenuates megakaryopoiesis via controlling the degree of phosphorylation of Akt.

Aims: The aim of this study was to unravel the role of PEAR1 in EC biology.

Methods: We compared the expression of PEAR1 in human endothelium of various tissues and determined its role for EC function *in vitro* and for angiogenesis in *Pear1*^{-/-} mice.

Results: PEAR1 is present on the membrane of human cultured ECs and it coincided with CD31 in various tissues. PEAR1-expression was variable in ECs of different origin. Lentiviral knockdown of *PEAR1* in cultured ECs by 70% doubled EC proliferation, in turn enhancing *in vitro* tube formation on matrigel through the Akt/PTEN-dependent p21/CDC2-pathway. Even when physiological blood vessel formation was unaffected in *Pear1*^{-/-} mice, neoangiogenesis in these mice was significantly increased both in a hind limb ischemia ligation model (4.7-fold increase in capillary density in the ligated limb of *Pear1*^{-/-} mice compared to ligated limbs in WT mice) and in a skin wound healing model (resulting in a 2-fold faster wound closure in *Pear1*^{-/-} mice compared to WT littermates).

Conclusion: We established an inverse correlation between endothelial PEAR1-expression and EC proliferation driven vascular assembly both *in vitro* and *in vivo*. These findings identify PEAR1 as a novel modifier of neoangiogenesis.

Disclosure of Interest: None declared.

ADAMTS-13

AS177

ADAMTS13: dynamic interactions with VWF

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Dynamic interactions control the outcome of encounters between VWF and its cleaving protease, ADAMTS13. Contact between ADAMTS13 and globular (folded) VWF, initially believed to be a positioning interaction, has now been shown to induce a conformational change in ADAMTS13. When the surface orientated VWF D4CK domains engage the distal TSP and CUB domains of ADAMTS13, the protease is induced to unfold from a compact structure mediated by a Spacer-CUB domain interaction, into an active conformation. In this active conformation, the functional Spacer domain exosite is unmasked and thereby made available for interaction with its cryptic VWF A2 domain complementary exosite. The resulting conformational activation of ADAMTS13 reveals the dominant auto antigenic determinant recognised by autoantibodies responsible for acquired TTP. The VWF substrate undergoes shear driven dynamic interactions that initiate its cleavage by ADAMTS13. The stabilising structural features controlling the unfolding of the VWF A2 domain have been identified and characterised. A vicinal Cys disulphide bond at its C-terminus provides an extractable hydrophobic plug. An intradomain Ca²⁺ binding site provides strand cohesion. A third stabilising element of the VWF A2 domain is the N-glycan at position 1574, which independently provides resistance to unfolding. Increasing shear progres-

sively disrupts these stabilising elements, exposes the cryptic (ADAMTS13) Spacer, Cys-rich and Disintegrin domain complementary exosites on the unfolded VWF A2 domain, allowing tight binding of conformationally activated ADAMTS13 and orientation of the VWF scissile bond over the catalytic centre of its MP domain. Collectively, these dynamic interactions provide a framework for understanding of VWF regulation by ADAMTS13 and suggest how protease action could be enhanced for therapeutic purposes.

Disclosure of Interest: None declared.

AS178

ADAMTS13-induced unfolding of the von Willebrand factor A2 domain

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Background: Distal (T2-CUB2) ADAMTS13 domains are crucial for binding to folded VWF. When VWF unfolds due to high shear, high affinity binding sites for the proximal (MDTCS) domains are exposed, promoting cleavage. The exosites in the distal domains involved in VWF binding have not been elucidated.

Aims: Identification of crucial distal domain(s) in the ADAMTS13-VWF interaction.

Methods: ADAMTS13 binding to folded VWF was assayed by ELISA. VWF was captured with an anti-VWF monoclonal antibody (mAb). ADAMTS13 (FL-ADAMTS13) or variants were added and detected with anti-ADAMTS13 mAbs. Proteolysis of folded VWF was studied under static non-denaturing conditions.

Results: The T8 domain was shown to comprise the major binding site for folded VWF by using ADAMTS13 variants with individual domain deletions. Compatible with this, partial inhibition of ADAMTS13 binding ($79 \pm 6\%$, $n = 5$) was observed with an anti-T8 mAb. Unexpectedly, an anti-spacer mAb also inhibited ADAMTS13 binding ($\leq 31 \pm 2\%$, $n = 3$). This was not due to steric hindrance because a FL-ADAMTS13 variant with point mutations in the spacer domain that abolish VWF A2 binding, also showed reduced bind to folded VWF ($\leq 16 \pm 6\%$, $n = 3$). Hence, ADAMTS13 binding to folded VWF via its distal domains induces a conformational change that exposes the spacer binding site in the VWF A2 domain. This was supported by reduced binding of FL-TS13 ($35 \pm 11\%$ compared to FL-VWF, $n = 3$) when a disulfide bridge-locked A2 domain variant VWF-CC2 was used. Interestingly, when folded VWF was incubated with MDTCS for 5 to 16 h, no proteolysis occurred (due to the folded structure). However, when FL-ADAMTS13 was added, VWF proteolysis was observed (for 5 and 16 h respectively 2.8 ± 1.2 and 3.1 ± 0.4 -fold increase, $n \geq 5$), indicating ADAMTS13-induced unfolding of the A2 domain, allowing proteolysis.

Conclusion: Binding of ADAMTS13 to folded VWF via its distal T8 domain leads to unfolding of the VWF A2 domain and subsequent cleavage.

Disclosure of Interest: None declared.

AS179

N-linked glycosylation is a modulator of ADAMTS13 expression, structure and function

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Background: ADAMTS13 (AD13) is a glycoprotein that regulates the multimeric size, therefore haemostatic potential of Von Willebrand Factor (VWF).

Aims: We aimed to investigate the effect of AD13 N-linked glycans (NLGs) on its proteolytic activity.

Methods: Wild-type AD13 and NLG variants were expressed in HEK293T cells and purified. Full length (FL) AD13 or AD13 containing the metalloprotease through spacer domain (MDTCS) with NLGs or terminal sialic acid (SA) removed by enzymic digestion were also used. The ability of the AD13 molecules to cleave VWF was assessed under static and flow conditions. Binding was determined using static phase binding assays and immunoprecipitation.

Results: Removal of terminal SA or entire NLGs from both FL-AD13 and MDTCS reduced cleavage of small AD13 substrates (FRET-76, VWF-A2 domain) and significantly reduced cleavage of FL-VWF under flow. Intriguingly, while PNGaseF treatment of FL-AD13 significantly decreased its binding to VWF-A2 domain; removal of NLGs from MDTCS did not affect VWF binding. We hypothesise that NLG modulate the recently determined intermolecular interaction within AD13, and their absence alters AD13 conformation in solution. To investigate this further we assessed proteolytic activity of AD13 devoid of NLGs in the CUB domains. Interestingly, mutations N1235Q and N1354Q enhanced the ability of AD13 to interact and proteolyse VWF under static and shear stress conditions.

Conclusion: We propose that the NLGs in the CUB domains of AD13 interact with the spacer domain and help to maintain the closed conformation of AD13. These data highlight the importance of AD13 NLGs for synthesis, secretion and maintenance of AD13 structure and their ability to modulate the interaction with VWF. The description of new gain of function mutants may be of therapeutic use. Furthermore, since the removal of terminal SA residues directly affects AD13 function further investigation is warranted into pathological states such as sepsis, where protein SA content may be altered.

Disclosure of Interest: None declared.

AS180

ADAMTS13 deficiency worsens murine colitis and treatment of wild type colitic mice with rhADAMTS13 leads to improvement

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Background: Inflammatory Bowel Disease (IBD) is a disease of chronic intestinal inflammation affecting 1.8 million people in the U.S. Patients with IBD are at three fold higher risk of thromboembolism, and this risk rises to 15 fold with active disease. Von Willebrand factor (VWF) is released from activated endothelium and binds both platelets and leukocytes. Thus VWF is both pro-thrombotic and pro-inflammatory. ADAMTS13 is a matrix metalloprotease which cleaves highly adhesive ultra large VWF. Patients with IBD have elevated plasma VWF, which rises further with disease activity.

Aims: To determine if ADAMTS13^{-/-} mice have worse colitis than wild type (WT). To investigate if treatment of WT colitic mice with recombinant human ADAMTS13 (rhADAMTS13) improves colitis.

Methods: Dextran Sodium Sulfate (DSS) in drinking water induces acute colitis in mice as DSS is toxic to gut epithelium. DSS colitis was induced in ADAMTS13^{-/-} and WT mice. Two additional groups of WT mice were given DSS and treated with rhADAMTS13 or vehicle control.

Results: ADAMTS13^{-/-} mice had more severe colitis compared to WT based on clinical colitis scores, histologic scoring of colonic tissue ($P < 0.05$), weight loss, and anemia ($P < 0.0005$). Colonic sub-mucosal vessels of ADAMTS13^{-/-} mice with colitis had one or more VWF-rich thrombi whereas none were observed in control tissue ($P < 0.02$). Colonic tissue from WT colitic mice had increased areas of intra and extra vascular VWF by fluorescent staining ($P < 0.005$) compared to control mice and this tended to increase further with ADAMTS13 deficiency ($P = 0.05$). Plasma VWF increased with colitis, more so in WT compared to ADAMTS13^{-/-} mice ($P < 0.005$). rhADAMTS13 treatment of WT mice with DSS colitis decreased weight loss, clinical colitis scores, and plasma IL-6 ($P < 0.03$), but did not worsen anemia ($P = 0.62$).

Conclusion: rhADAMTS13 may be of utility as an anti-inflammatory and antithrombotic agent in colitis which does not worsen anemia.

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AS181

Von Willebrand factor, ADAMTS13 and the risk of mortality: the rotterdam study

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Background: Von Willebrand Factor (VWF) is a protein that plays a major role in platelet adhesion and aggregation. Large VWF multimers are cleaved into smaller, less coagulant forms by the metalloprotease ADAMTS13. Previous studies have shown that VWF and ADAMTS13 are associated with the risk of ischemic stroke and myocardial infarction, but it is not yet known what the relationship is between these factors and mortality.

Aims: To prospectively establish the relationship between VWF levels, ADAMTS13 activity, and all-cause and cause specific mortality.

Methods: We included 6511 participants of the Rotterdam Study, a population-based cohort study among individuals ≥ 55 years. ADAMTS13 activity was measured by FRET-VWF73 and VWF:antigen (VWF:Ag) levels by ELISA. We determined the association between these levels and mortality by Cox proportional hazard regression analysis. Informed consent was obtained and the study was approved by a medical ethics committee.

Results: Over a median follow-up time of 11.3 years, 1868 individuals died (30.5%), of whom 442 due to cardiovascular disease (7.2%). In individuals with VWF:Ag levels in the highest quartile, the risks of all-cause mortality (HR 1.21, 95% CI 1.06–1.38) was higher than in those in the lowest quartile. For cardiovascular mortality the risk was 1.29 (95% CI 0.98–1.70). In individuals with the lowest ADAMTS13 activity, the risks of all-cause mortality and cardiovascular mortality were higher than in those with the highest ADAMTS13 activity (HR 1.46, 95% CI 1.26–1.69; HR 1.46, 95% CI 1.09–1.96; respectively). The risks of all-cause and cardiovascular mortality were even higher in individuals with both low ADAMTS13 activity and high VWF:Ag levels. ADAMTS13 activity and VWF levels were not associated with the risk of cancer and COPD related mortality.

Conclusion: In this large prospective cohort study, we show that VWF:Ag levels and ADAMTS13 activity are both associated with an increased risk of all-cause mortality and cardiovascular mortality.

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Novel anti-platelet agents

AS182

Chemoproteomic discovery of novel anti-platelet targets

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We previously used a chemoproteomic strategy in human platelets to identify a novel lipid hydrolase, arylacetamide deacetylase-like 1 (AADACL1) that contributes to platelet aggregation and secretion. Here we tested the hypothesis that AADACL1 regulates platelet function by controlling the levels of its major lipid substrate or deacetylated product. Since our previous evidence suggests that AADACL1 modulates platelet reactivity at or near the level of PKCs, we asked whether these lipids bind directly to the lipid-binding C1 domains of specific platelet PKC isoforms. We found that both the substrate, 2-acetyl MAGE, and the product, MAGE, in small unilamellar vesicles bound the PKCd C1b domain with high affinity, similar to the known PKC activator, diacylglycerol. However, only 2-acetyl MAGE, not MAGE inhibited PKCd kinase activity *in vitro*. Similarly, in agonist-stimulated platelets, only exogenously added 2-acetyl MAGE but not MAGE inhibited platelet aggregation and secretion. Thus, these data reveal a novel regulatory mechanism by which AADACL1 deacetylation of 2-acetyl MAGE relieves an endogenous inhibitory constraint on PKC in platelets.

Disclosure of Interest: None declared.

AS183

A small molecule inhibitor of ASK1 protects mice from thrombosis with minimal effect on hemostasis

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Background: Apoptosis signal-regulating kinase (ASK1) is a serine/threonine kinase, which is activated in response to stress. Role of ASK1 in platelet function and thrombosis is not known.

Aims: To evaluate the effect of ASK1 inhibitor on platelet function and thrombosis.

Methods: Phosphorylation of ASK1 and p38 mitogen activated protein kinase (MAPK) were assessed by Western blotting. Tail bleeding time was used to assess hemostasis. FeCl₃-induced injury and pulmonary thromboembolism in C57/BL6 mice were performed to assess thrombosis.

Results: We found that ASK1 is expressed in platelets and is rapidly activated by physiological agonists. Genetic ablation of Ask1 in mice results in severe anti-thrombotic phenotype. Lack of Ask1 affects granular secretion, thromboxane A2 generation, as well as integrin outside-in signaling. *N*-(6-(1H-imidazol-1-yl)imidazo[1,2-a]pyridin-2-yl)-4-(tert-butyl)benzamide, (IPTB) is a novel and highly specific inhibitor of ASK1 (Kd of 14 nM). We found that in human platelets, IPTB dose-dependently inhibited activation of ASK1 and its downstream effector p38 MAPK induced by 0.1U of thrombin. IPTB dose-depen-

dently inhibited ADP- and PAR4 peptide-induced platelet aggregation. Furthermore, IPTB dose-dependently inhibited platelet spreading on immobilized fibrinogen and fibrin clot retraction suggesting an effect on both inside-out and outside-in signaling. Additionally, IPTB dose-dependently delayed time to occlude vessel in FeCl₃-induced carotid artery injury model. IPTB (100 µg kg⁻¹) completely rescued mice from pulmonary thromboembolism induced by intravenous injection of collagen and epinephrine. Interestingly, IPTB had no effect on tail-bleeding time even at a dose as high as 1 mg kg⁻¹.

Conclusion: Our results strongly suggest that IPTB could attenuate thrombosis with a minimal effect on hemostasis. This development would make IPTB a novel potential therapeutic agent to be used to combat thrombotic disorders with minimal side effects of bleeding.

Disclosure of Interest: None declared.

AS184

Site specific inhibition of thrombus formation using shear sensitive anti-thrombotic nanoparticles

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Background: Anti-platelet therapy, used to treat thrombosis after atherosclerotic plaque rupture, can result in fatal bleeding complications because it is not tailored to act exclusively at sites of pathological thrombus formation where blood shear stresses are typically very high.

Aims: To site specifically inhibit thrombus formation, using flow sensitive nanoparticles loaded with anti-platelet drugs, without causing systemic platelet inhibition.

Methods: Anti-platelet drugs were used as proof of principle to demonstrate the feasibility of localized drug delivery induced by high blood shear stress. Cangrelor or eptifibatide were encapsulated in shear sensitive liposomes. Inhibition of thrombus formation by drug encapsulated nanoparticles was tested in microfluidic blood perfusion channels and *in vivo* in the carotid artery and mesenteric arterioles of mice.

Results: Nanoparticle delivery of anti-platelet drugs inhibited *in vitro* thrombus formation at sites of stenotic plaque geometries, i.e. at blood shear rates > 1000 s⁻¹. Infusion of nanoparticles in mice prevented full thrombotic occlusion. Live microscopy analysis revealed that the initial phases of thrombus build-up were unaffected. Mice infused with anti-platelet nanoparticles did not display increased tail bleeding times and platelets isolated from these mice aggregated normally in response to 10 µM ADP indicating an absence of systemic platelet inhibition.

Conclusion: Targeted delivery of anti-platelet drugs by flow sensitive nanoparticles offers a potent and site-specific therapy to prevent atherothrombotic events. The drug is only released in areas of pathological shear stress caused by the formation of platelet thrombi and atherosclerotic plaques but leaves other areas of the vasculature unaffected. This study is a step towards safer and more potent therapy inhibiting thrombosis in the setting of atherosclerosis and could be extended towards local delivery of fibrinolytics, anti-inflammatory, anti-proliferative or lipid-lowering drugs.

Disclosure of Interest: None declared.

AS185

Modulating platelet reactivity through regulated control of free RGS18 levels

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Background: We have shown that the Regulator of G protein Signaling family members expressed in platelets, including RGS18, can limit

signaling by agonists such as thrombin. We have also shown that in resting platelets RGS18 is bound to the scaffold protein, spinophilin (SPL), forming a ternary complex with SHP-1 that dissociates when platelets are activated.

Aims: Here we report a method for measuring free RGS18 levels in human platelets and use it to test ideas about the role of RGS18 in resting, activated and PGI₂-desensitized platelets.

Methods: Free RGS18 was measured with a novel pull-down assay. Spinophilin variants were studied in CHO cells and then tested in platelets by retroviral transduction of SPL^{-/-} fetal liver cells reconstituted in irradiated C57Bl/6 mice.

Results: The data show an increase in free RGS18 in thrombin-stimulated platelets, and an even greater increase following exposure to PGI₂. The latter is due to cAMP-dependent phosphorylation of spinophilin Ser94, which causes dissociation of RGS18 from spinophilin. Substitution of Asp (but not Ala) for Ser94 mimics Ser94 phosphorylation, prevents formation of the SPL/RGS18/SHP-1 complex and inhibits thrombin-induced changes in cytosolic Ca⁺⁺ in CHO cells. Reconstitution studies in mice with WT Vs. S94D spinophilin show a shift in the thrombin dose/response curve in S94D platelets such that higher concentrations of the agonist were required to cause aggregation.

Conclusion: These results validate the idea that free RGS18 levels rise during platelet activation and affect platelet reactivity. They also show for the first time that the desensitized state found in platelets exposed to endothelial PGI₂ is due in part to rising free RGS18 levels when phosphorylation of SPL Ser94 causes dissociation of the SPL/RGS18/SHP-1 complex. Finally, the results provide direct evidence that dissociation of RGS18 from spinophilin inhibits platelet activation by thrombin.

Disclosure of Interest: None declared.

Glycobiology

AS186

Platelet glycobiology: the powers of posttranslational modification

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The human body produces and removes 10¹¹ platelets daily to maintain a normal steady-state platelet count. Platelet production must be tightly regulated to avoid spontaneous bleeding or arterial occlusion and organ damage. Therefore, multifaceted and complex mechanisms control platelet removal and production in physiological and pathological conditions.

Recent studies have highlighted the role of glycan modifications on platelet surface proteins in mediating platelet clearance. Platelets with reduced α2,3-linked sialic acid, during sepsis, after cold storage, or in mice lacking the sialyltransferase ST3GalIV, are cleared by the hepatic endocytic Ashwell-Morell receptor (AMR). The AMR is a transmembrane heterooligomeric glycoprotein complex composed of ASGPR1 (CLEC4H1, HL-1) and ASGPR2 (CLEC4H2, HL-2) subunits, which are highly conserved among mammalian species. However, since its discovery four decades ago, the regulatory role of the hepatic AMR has remained unclear.

New evidence shows that the circulatory lifespan of platelets is determined by sialic acid loss that triggers platelet removal by the hepatic AMR. This removal system drives hepatic thrombopoietin (TPO) mRNA expression *in vivo* and *in vitro* by recruiting Janus kinase 2 (JAK2) and the acute phase response signal transducer and activator of transcription 3 (STAT3) to increase thrombopoiesis. Disruption of AMR-desialylated platelet signaling by JAK1/2 inhibitors adversely affects hepatic TPO mRNA expression and secretion. This feedback mechanism posits the AMR-desialylated platelet pair as the critical

control point for TPO homeostasis and contributes to our understanding why JAK1/2 inhibition commonly induces thrombocytopenia in patients with Myeloproliferative Neoplasms. Furthermore, the recognition of this novel physiological feedback mechanism illuminates the pathophysiology of platelet diseases, such as Essential Thrombocythemia and Immune Thrombocytopenia.

Disclosure of Interest: None declared.

AS187

Recessive and transient antithrombin deficiency caused by a wide spectrum of congenital disorders of N-glycosylation with unusual clinical features.

Identification of a new thrombophilic disorder

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Background: Antithrombin (AT) deficiency, the strongest thrombophilic factor, is mainly caused by *SERPINC1* mutations, but up to 25% have unknown cause.

Aims: To identify new elements and mechanisms involved in AT deficiency associated with thrombosis.

Methods: We study 30 patients with AT deficiency and thrombosis but no *SERPINC1* defects selected from 139 cases. AT, antitrypsin and transferrin glycoforms were evaluated by electrophoresis, HPLC and Q-TOF. Genetic analysis included sequencing of candidate genes and whole exome analysis.

Results: Eight patients (27%) had increased AT glycoforms with reduced content of N-glycans. The hypoglycosylation also affected all tested N-glycoproteins, with a type 1 pattern, indistinguishable from that of PMM2-CDG, the most frequent congenital disorder of glycosylation (CDG), a rare recessive disorder described in children with mental retardation. Three cases had permanent AT deficiency: P1, with mental retardation, was *PMM2* compound heterozygous (F119L&R141H). P2 carried an *ALG12* homozygous mutation (V26D). She is the 8th *ALG12*-CDG patient described worldwide. P3 had a heterozygous mutation in *PMM2* (R141H) and a profile of heterozygous SNPs in other genes involved in the N-glycosylation pathway (*SLC5A9*, *ALG6* and *PGM1*), which in family analysis associated with hypoglycosylation and AT deficiency. P2&3 have no mental retardation. In the remaining cases (4–8), without clinical signs except for thrombosis, AT deficiency and hypoglycosylated glycoforms were transitory. Interestingly, P4–P8 reported moderate/high alcohol consumption, a factor that also impairs N-glycosylation. P4–P8 only had a single heterozygous mutation in *PMM2*, *IDI2* or *SLC5A9*.

Conclusion: An aberrant N-glycosylation caused by a myriad of different mechanisms and genes affected underlies a high proportion of cases with AT deficiency, but no *SERPINC1* mutations. We identified new and fascinating CDGs cases, supporting that this rare disorder may be underestimated and should be considered as a new thrombophilic disorder.

Disclosure of Interest: None declared.

AS188

Mechanism of N-linked glycan stabilisation of the von Willebrand factor A2 domain

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Background: Shear forces in the blood trigger a conformational transition in the VWF A2 domain, from its native folded to an unfolded state, in which the cryptic scissile bond is exposed and is proteolysed by ADAMTS13. The conformational transition depends upon a Ca²⁺ binding site (CBS) and a vicinal cysteine disulphide bond (VicCC). N-linked glycosylation of VWF A2 domain at N1574 has also been shown to confer resistance to proteolysis, but with an uncertain mechanism as it is positioned away from known ADAMTS13 binding sites. **Aims:** To investigate the mechanism by which N-linked glycosylation protects the VWF A2 domain from cleavage by ADAMTS13.

Methods: The VWF A2 domain and ADAMTS13 were expressed in mammalian cells. Thermal stability/unfolding (T_m) of VWFA2 was measured with SyproOrange. ADAMTS13 binding and cleavage were performed using established assays.

Results: Complete removal of glycan at N1574 (by mutagenesis, N1574Q, or PNGase treatment) destabilised the VWF A2 domain, ΔT_m 6–10 °C. Glycan stabilisation was quantitatively similar to that provided by CBS and VicCC and was cooperative. In contrast, mutation N1515Q, reduction and/or selective trimming using EndoH treatment of the VWFA2 glycan structure (to the residual GlcNAc residue) caused no reduction in stability. Destabilisation of the VWF A2 domain directly revealed ADAMTS13 binding site(s), with a strong correlation, r = -0.96, between T_m of VWF A2 variants and their solution binding (%).

Conclusion: Glycosylation at N1574 has previously been suggested to modulate VWF A2 domain interaction with ADAMTS13 through steric hindrance by the bulky carbohydrate structure. Here we show that only the first sugar residue (N1574-GlcNAc) is required to protect VWF A2 from ADAMTS13 proteolysis by providing stability to the VWF A2 domain. We propose that N1574-GlcNAc participates in an intradomain stabilising interaction. This stabilising interaction helps to maintain the folded structure of VWF A2 and restricts ADAMTS13 binding and cleavage of the scissile bond.

Disclosure of Interest: None declared.

AS189

Peptide antagonists for P-selectin discriminate between sulfatide-dependent platelet aggregation and PSGL-1-mediated cell adhesion

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Background: Membrane-exposed sulfatides contribute to P-selectin-dependent platelet aggregation by binding P-selectin, thereby stabilizing platelet aggregates. This may explain the slight bleeding tendency observed in P-selectin-deficient mice and indicates that development of antagonists of the sulfatide/P-selectin interaction may benefit in the treatment of acute coronary symptoms.

Aims: To characterize the molecular mechanism underlying the P-selectin/sulfatide interaction and its implications for P-selectin-directed intervention in thrombosis.

Methods: We designed specific peptide antagonists for P-selectin carrying an EWVDV minimal motif through which they interact specifically with P-selectin, and studied the impact of these peptides on platelet reactivity.

Results: Upon platelet activation, sulfatides are translocated to the platelet surface to form focal hot-spots. Correspondingly, using sulfatide-containing liposomes, we observed that P-selectin exclusively interacts with liposomes with a sulfatide density higher than 21% (w/w), indicating that the binding profile of P-selectin for sulfatide-rich liposomes is dependent on sulfatide density. Sulfatide-liposome binding to P-selectin was blunted by our specifically designed EWVDV-based peptide antagonists with N-terminal extensions such as CDVEWVDVSC ($IC_{50} = 0.2 \mu M$), but not by the EWVDV core motif itself ($IC_{50} > 1000 \mu M$), although both antagonists were equally potent inhibitors of the PSGL-1/P-selectin interaction ($IC_{50} = 7\text{--}12 \mu M$). Elongated peptides but not truncated peptides were found to inhibit aggregation of human platelets and reduce aggregate size. P-selectin-mediated platelet aggregation on a collagen-coated surface under flow also depended on sulfatides.

Conclusion: The sulfatide/P-selectin interaction implicates multiple binding pockets in P-selectin that only partly overlap with that for PSGL-1. These findings open ways to selectively interfere with sulfatide/P-selectin-dependent platelet aggregation without affecting PSGL-1-dependent cell adhesion.

Disclosure of Interest: None declared.

Inhibitors – basic

AS190

The immunogenicity of factor VIII

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Approximately thirty percent of patients with severe hemophilia A develop anti-factor VIII (FVIII) antibodies with associated increases in both morbidity and mortality. Because FVIII is an immunologically foreign protein or an altered self-protein in severe hemophilia A patients and FVIII^{-/-} mice, it may not seem surprising that intravenous infusions of the FVIII protein produce an antibody response. However, it usually is difficult to raise antibodies to an intravenously delivered foreign protein without using adjuvants. In addition, FVIII is the most commonly targeted coagulation factor in autoimmunity. FVIII inhibitor formation is a MHC Class II T cell-dependent process. Activation of naïve T-cells requires costimulatory molecules and potential 'danger' signals associated with FVIII have been investigated. FVIII alone or with its large carrier protein von Willebrand factor (VWF) is not sufficient to provide the costimulatory signal. Furthermore, given the crosstalk between coagulation and inflammation it was hypothesized that the procoagulant function of FVIII might itself be a 'danger' signal but studies have shown that immunogenicity of FVIII is independent of its procoagulant function. Since FVIII circulates in a non-covalent complex with VWF, VWF must be considered as a possible factor in its immunogenicity. Several studies have suggested that VWF decreases the uptake and presentation of FVIII to the immune system by antigen presenting cells *in vitro*, however, the nature of FVIII immune recognition *in vivo* is not known. There is on-going debate over whether plasma derived VWF containing FVIII products are less immunogenic than recombinant FVIII products in patients with hemophilia A. FVIII is an unusually immunogenic protein and the mechanisms of this immunogenicity continue to be a focus of considerable investigation.

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AS191

Chemotactic antigen competition decreases the incidence of factor VIII inhibitors in hemophilia a mice immunized with influenza, with and without the adjuvant MF59C.1

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Background: Inhibitory antibodies against factor VIII (FVIII) are the most important treatment complication in hemophilia A (HA). In the 25–30% of patients who develop inhibitors, concurrent inflammatory signals, such as vaccination, have been hypothesized as risk factors.

Aims: To assess the effect of influenza vaccination, with and without an adjuvant, on the development of FVIII antibodies and inhibitors.

Methods: F8E17KO HA mice with a humanized HLA-DRB1*1501 allele were infused with 6 IU of recombinant human FVIII 7 times biweekly. Intramuscular (IM) immunization, with or without the MF59C.1 adjuvant, occurred within 24 h of the initial FVIII treatment. FVIII and influenza-specific IgG was assessed by ELISA and Bethesda at week 5. Splenocyte chemotaxis, from F8E16KO Balb/C mice, was observed in FVIII or influenza-induced gradients.

Results: IM influenza immunization decreased the incidence of α FVIII IgG ($t = -24, 0, +24$ h: 30%, 27%, 14% vs. control: 67%; $P = 0.11, 0.06, 0.008$; $n = 10\text{--}15$) and inhibitors (30%, 43%, 11% vs. 80%; $P = 0.03, 0.02, 0.002$) at all time points. Similar results were observed with the addition of MF59C.1 in α FVIII IgG (50%, 19%, 42% vs. 67%; $P = 0.42, 0.01, 0.26$; $n = 11\text{--}16$) and inhibitor incidence (36%, 31%, 33% vs. 80%; $P = 0.04, 0.01, 0.02$). All mice developed high titre influenza-specific antibodies. No significant differences in IgG or Bethesda titres were observed. In IgG positive mice, mice exposed to influenza had increased levels of α FVIII IgG2a ($P = 0.04$).

Chemotaxis assays demonstrated that naïve splenocytes migrate preferentially over 3 h towards media from influenza- vs. FVIII-exposed splenocytes based on the distribution of migration endpoints with and without an adjuvant ($P = 0.014, 0.0018$ respectively).

Conclusion: IM immunization against influenza within 24 h, with and without an adjuvant, confers relative tolerance to FVIII. We propose that antigen competition via splenocyte chemotaxis contributes to this phenomenon.

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AS192

Factor VIII tolerance induction in haemophilia a mice via transplacental transfer of recombinant factor VIII Fc

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Background: The most severe complication of haemophilia A (HA) treatment is the development of inhibitory antibodies against factor VIII (FVIII). *In utero* FVIII exposure of HA fetuses could result in central tolerance to the protein. rFVIII-Fc, a recombinant fusion protein composed of a single B-domain deleted (BDD) FVIII molecule covalently fused to the Fc region of human immunoglobulin G1 (IgG1), may be able to undergo transplacental transfer due to its abil-

ity to interact with the neonatal Fc receptor, resulting in tolerance to FVIII in the progeny.

Aims: 1 Assess the ability of rFVIII-Fc to undergo transplacental transport in pregnant HA mice.

2 Determine if *in utero* exposure of HA mouse fetuses to rFVIII-Fc induces FVIII tolerance.

Methods: 1 Pregnant E16KO C57Bl6 HA mice received 376 IU rFVIII-Fc or rBDD-FVIII on day 19 of gestation. Fetal blood was collected 3 h later. FVIII activity in the fetal samples was determined using a chromogenic assay.

2 Pregnant E16KO C57Bl6 HA mice received 376 IU rFVIII-Fc or rBDD-FVIII on day 16 and 19 of gestation. Newborn mice were transferred to an E16KO Balb/c surrogate mother to avoid antigen transfer from milk. Once mature, mice were challenged with 2 IU rBDD-FVIII weekly for 4 consecutive weeks. 1 week after the last FVIII injection, blood was collected. Binding and neutralizing antibody titers to FVIII were determined using an ELISA and Bethesda assay respectively.

Results: 1 FVIII activity was detected in 9/10 fetal samples from rFVIII-Fc-treated mothers but 0/9 fetal samples from rBDD-FVIII-treated mothers.

2 Following FVIII challenge, 3/8 mice from rFVIII-Fc-treated mothers and 6/8 mice from rBDD-FVIII-treated mothers had developed anti-FVIII IgG. Similarly, 3/8 mice from rFVIII-Fc treated mothers and 5/8 from rBDD-FVIII-treated mothers had evidence of FVIII inhibitors.

Conclusion: rFVIII-Fc undergoes transplacental transfer in pregnant HA mice. The resultant exposure of HA fetuses to rFVIII-Fc *in utero* may offer protection from inhibitor development later in life.

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AS193

Can a 'center effect' be the reason for the higher frequency inhibitors for a second generation recombinant factor VIII product?

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Background: It has been hypothesized that preference for certain products in a hemophilia treatment center may be a confounder in multi-center studies on frequency of inhibitors in PUPs with severe hemophilia A.

Aims: To investigate a potential center effect on the higher inhibitor incidence for 2nd generation full length recombinant FVIII

Methods: A total of 604 PUPs with severe hemophilia A from 29 PedNet centers were available. Centers were divided into 2 groups; small centers that included < 15 patients and large centers that included ≥ 15 patients with severe hemophilia A.

Results: We identified 10 large centers with 354 patients (59% of patients) and 19 small centers with 250 patients (41%). Large centers had a higher frequency of inhibitors compared to small centers 34.5% vs. 24.0% ($P = 0.006$). This difference is mainly due to a higher number of low titer inhibitor patients diagnosed in the large centers. The number of high titer patients was 22.3% for the large centers and 17.2% for the small centers, which is not significantly different ($P = 0.123$). The frequency of testing in the first 50 EDS was also

higher for large centers: median of 5 inhibitor tests vs. 3 for smaller centers ($P = 0.002$). The percentage of patients that had a positive family history for inhibitors was not different. Patients that received intensive treatment at first treatment was similar with 15% and 16% respectively for large and small centers. However, the dose administered during these 5 EDS was 82 IU kg⁻¹ per ED for large centers and 68 IU kg⁻¹ per ED for small centers; this was statistically different ($P = 0.001$). Although large centers had a higher inhibitor incidence the usage of 2nd generation full length rFVIII product was 29.9% in large centers while 32.4% of patients in small centers used the 2nd generation full length rFVIII product.

Conclusion: No confounding 'center effect' was found that influenced the higher incidence for inhibitors on 2nd generation full-length rFVIII product.

Disclosure of Interest: None declared.

Unusual site venous thrombosis

AS195

Antithrombotic treatment and outcomes of cirrhotic patients with splanchnic vein thrombosis: a sub-study from the ISTH registry

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Background: Cirrhotic patients have often been excluded from studies addressing the anticoagulant treatment of splanchnic vein thrombosis (SVT), although liver cirrhosis is associated with an increased risk of SVT through hypercoagulability.

Aims: To assess the outcomes of cirrhotic patients from an unselected cohort of SVT patients.

Methods: International prospective registry of consecutive SVT patients, enrolled from 2008 to 2012. Therapeutic strategies and outcomes were analysed separately for cirrhotic patients. A Central Adjudication Committee reviewed and classified clinical outcomes as vascular events (venous or arterial thrombosis) and major bleeding (MB; ISTH definition plus the need for hospitalization).

Results: A total of 167 (28%) of 604 patients had liver cirrhosis (median age 59 years, 70.7% males, 79.6% portal vein thrombosis, 49.4% inci-

dentally detected SVT). Sixty-six patients (39.5%) received no anticoagulation; 62 received parenteral anticoagulants alone (median duration 6 months, IQR 3–15) and 39 were started on vitamin K antagonists (median 10 months, IQR 4–24). Median follow-up duration was 2 years (IQR 0.5–2); 5 patients (3.0%) were lost to follow-up. The overall incidence of recurrent thrombotic events was 11.3/100 patient-years (pt-y) (95% CI 7.7–16.8) and the incidence of MB was 10.0/100 pt-y (95% CI 6.6–15.1). The incidence of these two outcomes in never-treated cirrhotic patients was 14.1/100 pt-y and 11.3/100 pt-y, respectively. In multivariate analysis, anticoagulant treatment was associated with lower rates of both vascular events (HR 0.86, 95% CI 0.77–0.96) and MB (HR 0.83, 95% CI 0.69–0.99).

Conclusion: Our real-life data suggest that more than a half of cirrhotic patients with SVT receive anticoagulant treatment. The incidence of thrombotic and bleeding complications was not negligible in this subgroup of patients. In selected cirrhotic patients, the anticoagulant treatment, usually administered at adjusted doses according to the individual risk of bleeding, appeared to be beneficial.

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AS196

Next-generation DNA sequencing approach to identify novel genetic risk factors underlying cerebral vein thrombosis

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Background: Cerebral vein thrombosis (CVT) is a rare life-threatening disease affecting annually 4 adults per million. Transient risk factors for CVT include oral contraceptive use, pregnancy, trauma, brain tumors and local infections. Genetic risk factors are deficiencies of the natural anticoagulant proteins antithrombin, protein C, protein S, factor V Leiden and prothrombin 20210A mutation. In 20% of patients, the cause of CVT remains unknown.

Aims: To identify novel genetic risk factors underlying CVT using targeted next-generation DNA sequencing (NGS).

Methods: As part of Milan-Leiden Sequencing (MILES) study, we investigated 171 Italian CVT patients and 298 healthy controls. Patients were selected using the following criteria: objective diagnosis of CVT, European ancestry, no active cancer. We performed targeted NGS analysis of the protein-coding regions and 3' and 5' UTRs of 740 candidate genes related to hemostasis and inflammation, 150 ancestry informative markers and 28 thrombosis-associated variants with a final target size of approximately 4 Mb.

Results: We obtained 37588 variants that passed quality control, of which 35988 were single nucleotide variants and 1600 were insertions/deletions (indels), that were sequenced with a mean depth of 86. We further filtered the data to obtain 5309 common variants (minor allele

frequency [MAF] > 1%) and 19583 rare variants (MAF < 1%). Single variant association testing of the common variants using logistic regression analysis revealed rs8176719 indel variant in the *ABO* gene as a putative risk factor for CVT (age and sex adjusted $P = 1.37 \times 10^{-6}$; OR 2.04; 95% CI 1.53–2.73; false discovery rate 0.0073).

Conclusion: We have successfully applied NGS-based analysis of 740 candidate hemostatic and pro-inflammatory genes in 469 Italian CVT patients and controls, identifying a common indel variant rs8176719 in the *ABO* gene that has been previously associated with deep vein thrombosis of the lower limbs and pulmonary embolism.

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AS197

The current management strategies and clinical outcome of upper extremity deep vein thrombosis

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Background: The incidence of upper extremity deep vein thrombosis (UEDVT) is increasing. Several questions regarding optimal management and natural course of UEDVT remain unanswered.

Aims: Investigate the current treatment approaches and clinical outcome of UEDVT.

Methods: Follow-up data were collected from patients with confirmed UEDVT, enrolled from January 2010 to June 2012 in an international diagnostic management study (Kleinjan et al., Ann Intern Med, 2014).

Between August 2014 and January 2015, patients were interviewed by telephone and questioned about recurrent VTE, bleeding, mortality, and PTS signs.

Results: Follow-up was completed for 102 patients (98.1%), of which 42 (40.4%) patients with cancer and 44 (42.3%) with venous catheters. The median follow-up duration was 3.3 years (IQR 2.3 – 3.8). Anticoagulant treatment was started in 100 of 102 patients (98%). Low-molecular-weight heparin (LMWH) was the most prescribed initial type of treatment (86.2%), whereas for long-term treatment 54.9% received vitamin K antagonists (VKA) and 39.2% LMWH. The median treatment duration was 182 days (IQR 91–365). Major bleeding occurred in 3.9% of patients during anticoagulant therapy. Overall, 10.8% had recurrent VTE and 25.5% died. PTS signs of the arm were reported by 36.1% of the interviewed patients.

Conclusion: The results of this study provide insight in the current management and outcome of UEDVT. Patients are treated mostly for 6 months, and in parallel with leg DVT, PTS signs are common after UEDVT. Moreover, UEDVT appears to carry a substantial risk of serious consequences like recurrent VTE and early mortality.

Disclosure of Interest: None declared.

Atherosclerosis: progression and development

AS198

Endothelial dysfunction, inflammation and the initiation of atherosclerosis

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Disturbed laminar blood flow in arterial regions predisposed to atherosclerosis promotes low-grade inflammation and recruitment of blood monocytes, which adopt features of dendritic cells (DCs) in the intima. The function of intimal DCs in normal arteries remains unknown. Recently, we observed that systemic stimulation of Toll-like or NOD-like receptors induces CCR7-dependent reverse transmigration of intimal DCs through the endothelial monolayer into the arterial blood. This process protects the artery wall, since it is critical for transporting intracellular pathogens from the intima of infected normocholesterolemic mice. Upon initiation of dietary hypercholesterolemia in *Ldlr*^{-/-} mice, intimal DCs rapidly accumulate intracellular lipid and transform into the initial foam cells of nascent atherosclerotic lesions. This is the earliest stage of lesion formation. Deletion of intimal DCs immediately prior to the initiation of hypercholesterolemia results in a marked reduction of foam cells within the first week, and diminished intimal lipid accumulation. Persistent deletion of intimal myeloid cells in the setting of hypercholesterolemia results in abrogation of monocyte recruitment to early atherosclerotic lesions. This suggests that intimal myeloid cells are required for inflammation in the early stages of atherosclerosis, and that retention of lipoproteins in a myeloid cell-depleted intima is insufficient. Reduced local production of chemokines and endothelial cell adhesion molecules was found in mice with deleted intimal myeloid cells, which suggests that cross-talk between lipid-loaded intimal myeloid cells and the overlying endothelium promotes inflammation.

Disclosure of Interest: None declared.

AS199

Platelet-derived matrix metalloproteinase (MMP)-2 contributes to atherosclerosis progression in hypercholesterolemic mice

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Background: MMPs participate in atherosclerosis, inflammatory cell recruitment, neointima formation and plaque rupture. Platelets contain and release MMP-2. Activated platelets participate in atherogenesis but the mechanisms are not fully understood.

Aims: Aim of the present study was to evaluate if platelet-released MMP-2 plays a role in atherogenesis *in vivo*.

Methods: We generated double knockout mice lacking the LDL receptor and MMP-2 (dKO), and mice lacking the LDL receptor plus only platelet MMP-2 (chimera). Moreover, *LDLR*^{-/-} mice rendered thrombocytopenic were transfused with thrombin-activated platelets from control or MMP-2^{-/-} mice every 4 days for 8 weeks. All mice were fed an atherogenic diet for 12 weeks. Damage-induced femoral artery intimal hyperplasia and lipid-rich plaques in the aortic arch were measured. *In vitro* co-incubation experiments with mouse platelets and human endothelial cells (EC) were also conducted.

Results: Femoral artery intimal hyperplasia was significantly lower in dKO and in chimeric mice compared with *LDLR*^{-/-} mice (IM ratio: *LDLR*^{-/-} = 1.6 ± 0.4; dKO = 0.42 ± 0.2, chimeras = 0.12 ± 0.02, *P* < 0.001). Similarly, aortic arch area covered by lipid lesions was lower in dKO and in chimeric mice than in *LDLR*^{-/-} mice (29 ± 3.2%, 21 ± 7.3% vs. 72 ± 5%, *P* < 0.01).

Aortic atherosclerotic area of *LDLR*^{-/-} mice infused with activated platelets from MMP-2^{-/-} mice was strikingly reduced (*LDLR*^{-/-} not infused: 15.2 ± 2%; *LDLR*^{-/-}-control platelets = 23 ± 5%; *LDLR*^{-/-}-MMP-2^{-/-}platelets: 4.8 ± 0.71%). Thrombin-activated platelets from *LDLR*^{-/-} mice, but not from MMP-2^{-/-} mice, induced EC activation (VCAM-1 expression: 4.4 and 1.7 fold increase vs. baseline, *P* < 0.001).

Conclusion: MMP-2 expressed/released by activated platelets plays a central role in hyperlipidemia-induced atherogenesis by a mechanism that involves EC activation. The interference with the expression/release of MMP-2 by platelets may represent a new target for anti-atherogenic therapy.

Disclosure of Interest: None declared.

AS200

Macrophage M2 differentiation induces PAI-1 expression via a STAT6 dependent pathway

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Background: Macrophages are a heterogeneous cell population. Depending on their priming they can be separated into proinflammatory M1 and anti-inflammatory M2 macrophages.

Aims: To determine if macrophage subsets have different proteolytic capacity via the expression of plasminogen activator inhibitor-1 (PAI-1).

Methods: Monocytes were cultivated for 7d with MCSF to generate M0 macrophages. Macrophages were polarized by LPS and interferon-gamma to generate M1 and by interleukin-4 (IL4) and IL13 to generate M2 macrophages. PAI-1 expression was determined by qPCR and specific ELISA.

Results: Polarization of macrophages was determined by measuring key markers for macrophage differentiation. After polarization M1 showed

a signature of high CD80, TNF, IL1 and IL6 expression whereas M2 were identified by high levels of CD206 and IL10. We observed different capability of matrix degradation indicating different level of proteolytic activity. Whereas M1 degraded 8.8% of gelatin matrix, M2 only degraded 1.5% ($P = 0.003$). In comparison, M0 showed 1.3% degradation capacity. The different proteolytic activity could stem from a differential availability of PAI-1. We therefore measured PAI-1 protein levels 48 h after polarization. M2 had the highest PAI-1 levels (34.8 ng mL^{-1}) compared to M1 (4.9 ng mL^{-1}) and M0 (2.2 ng mL^{-1}). This PAI-1 induction was already detectable after 2 h at the mRNA level (2 fold upregulation vs. M0 and M1). Time course experiments revealed the maximum protein induction was already reached after 24 h. The main signal transduction pathway of IL4 and IL13 is the activation of STAT6. A bioinformatics analysis of the PAI-1 promoter found an undescribed STAT6 element. Inhibiting STAT6 abrogated IL4 and IL13 induced PAI-1 expression. In addition, M2 were the only subset to upregulate PAI-1 expression after IL1 stimulation.

Conclusion: M2 polarized macrophages display reduced proteolytic activity associated with a STAT6 dependent induction of PAI-1 and remain active PAI-1 producing cells under inflammatory conditions.

Disclosure of Interest: None declared.

AS201

M1 and M2 macrophage proteolytic and angiogenic profiles analysis in atherosclerotic patients reveals a distinctive profile in type 2 diabetes

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Background: Diabetics present high risk of cardiovascular morbidity, associated to more extensive and vulnerable atherosclerotic plaques. Two macrophage subpopulations, M1 (classically activated macrophages) and M2 (alternatively activated macrophages), have been identified in human atherosclerotic plaques. The mechanisms that promote accelerated atherosclerosis in diabetes are poorly understood. **Aims:** To investigate the expression levels of atherosclerotic mediators in M1 and M2 macrophages and to focus on the influence of diabetes on M1/M2 profiles.

Methods: Monocyte-derived macrophages from 36 atherosclerotic patients (19 diabetics and 17 non-diabetics) were cultured with IL-1 β (10 ng mL^{-1}) or IL-4 (10 ng mL^{-1}) to induce the M1 or M2 phenotype, respectively. The expression of atherosclerotic mediators was evaluated by quantitative RT-PCR. Carotid plaques from these patients were histologically characterized.

Results: M1 and M2 macrophages differentially expressed mediators involved in proteolysis and angiogenesis processes. The proteolytic balance (MMP-9/TIMP-1, MMP-9/PAI-1 and MMP-9/TFPI-2 ratios) was higher in M1 vs. M2, whereas M2 macrophages presented higher angiogenesis properties (increased VEGF/TFPI-2 and TF/TFPI-2 ratios). Moreover, our results revealed that M1 macrophages from diabetics compared to non-diabetics displayed more important proangiogenic and proteolytic activities. These new findings may explain increased neovascularization in diabetics (increased microvessels content and VEGF staining). Finally, diabetes was not associated to changes in plaque content of M1 and M2 macrophages.

Conclusion: The present study reveals that M1 and M2 macrophages could differentially modulate major atherosclerosis-related pathological processes. Moreover, M1 macrophages from diabetics display a deleterious phenotype that could explain the higher plaque vulnerability observed in these subjects.

Disclosure of Interest: None declared.

AS202

Identification of cardiovascular disease specific genes and their regulating miRNA's

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Background: Identification of differentially expressed genes in diet induced atherosclerosis using mouse model facilitates the understanding of the progressive disease development. We used Apob^{tm2Sgy} Ldlr^{tm1Her}/mice to study the gene expression at different stages of lesion progression. Classification of genes based on their function and identification of specific genes related to cardiovascular disease is crucial for understanding their role in atherosclerosis pathology.

Aims: To study the differentially regulated genes involved in early and intermediate stage of the cardiovascular disease, to identify disease related genes and gene-miRNA interaction.

Methods: Global gene expression was performed with RNA extracted from ascending aorta with atherosclerotic lesion at 4, 8, 14 and 20-week time points. Samples were hybridized in Agilent 8 × 60K mouse gene expression array slides and scanned. Enrichment of gene clusters based on the early and intermediate stage was done using DAVID annotation tool. CVD specific genes were picked up using RGD database. The TF-gene-miRNA network was constructed using cytoscape.

Results: We clustered the genes expressed in Early and Intermediate stage of the disease. The main clusters enriched were MMPs, Proliferation differentials, ECM differentials and Adhesion differentials. About 16 genes were shortlisted and network was constructed with their interacting miRNAs. MicroRNA's mmu-miR-484 and mmu-miR-335-5p were found to interact with maximum number of genes. The genes targeting mmu-miR-484 are LRAT, CSF2RB2, SAA4, NCAN, MEFV, BST1, FCGR4 and SLC6A13. The genes targeting mmu-miR-335-5p are LRAT, HHIP, CSF2BR2, CYP11a1, CCL7, SAA4, NCAN and MEFV. Five genes LRAT, CSF2RB2, SAA4, NCAN and MEFV are targeting both miRNAs rendering these genes to be important ones.

Conclusion: This data identifies two novel miRNA interacting with cardiovascular disease specific genes which could be potential targets for cardiovascular therapy.

Disclosure of Interest: None declared.

Fibrinolysis in health and disease

AS203

Roles of Plg-Rkt in health and disease

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When plasminogen binds to cells its activation is markedly enhanced compared to the reaction in solution. Arming cells with the proteolytic activity of plasmin is a key feature in physiological and pathological processes in which cells must degrade extracellular matrices in order to migrate. We have identified Plasminogen Receptor KT (Plg-R_{KT}), a novel transmembrane plasminogen receptor, which binds plasminogen via a carboxyl-terminal lysine. Plg-R_{KT} is broadly expressed on leukocytes, epithelial cells, neuroendocrine tissue, the vasculature, epidermis and liver. In order to evaluate the role of Plg-R_{KT} *in vivo* we generated Plg-R_{KT}^{-/-} mice using homologous recombination and backcrossed the mice into the C57BL/6J background. Plasminogen binding to Plg-R_{KT}^{-/-} macrophages was only 20% of that of Plg-R_{KT}^{+/+} macrophages. Furthermore, when plasminogen was incubated with Plg-R_{KT}^{-/-} cells the rate of plasminogen activation was markedly reduced. These results suggest that Plg-R_{KT} is a major regulator of cell surface

plasminogen activation. Additional spontaneous phenotypes observed in Plg-R_{KT}^{-/-} mice and results of disease models will be discussed.

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AS204

Plasminogen localizes to distinct caps on phosphatidylserine-exposing platelets and promotes thrombus lysis under flow

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Background: Plasminogen interacts with both platelets and fibrin, but its localization and distribution in thrombi during fibrinolysis under flow is not well defined.

Aims: To characterize plasminogen on platelets and in thrombi and examine fluctuations during lysis under flow.

Methods: We established a novel model of whole blood thrombus formation to analyze fibrinolysis using fluorescence microscopy. Plasminogen binding and localization on the platelet surface was analyzed by flow cytometry and confocal microscopy.

Results: Thrombus lysis was accelerated by flow, irrespective of the shear rate, and was dependent on the plasminogen activator and its concentration. Heparin enhanced fibrinolysis indicating concurrent fibrin formation and degradation. Fibrin fibers distal to platelet aggregates were degraded first, whilst fibrin adjacent to platelet aggregates showed increased resistance to tPA-mediated lysis. Fluorescently-labeled plasminogen radiates from platelet aggregates at the base of thrombi, primarily co-localizing with fibrin. In line with this, hirudin attenuates, but does not abolish, plasminogen binding. Activation of washed platelets with thrombin/convulxin significantly augments plasminogen binding which is primarily associated with phosphatidylserine (PS)-exposing platelets. Binding is down-regulated by tirofiban and Gly-Pro-Arg-Pro amide, emphasizing a key role for fibrin in anchoring plasminogen to PS-exposing platelets. Binding of plasminogen and fibrin(ogen) co-localized with PAC-1 antibody, a marker of active αIIbβ₃, in the center of spread platelets. In contrast, PS-exposing platelets were PAC-1 negative, but demonstrated bound plasminogen and fibrinogen in a distinct protruding 'cap' on their membrane surface.

Conclusion: Different subpopulations of platelets harbor plasminogen by diverse mechanisms and provide a vital platform for accrual of fibrinolytic proteins that subsequently mediate fibrinolysis under flow.

Disclosure of Interest: None declared.

AS205

Inhibition of TAFI affects AAA formation and rupture in the angiotensin II model of abdominal aortic aneurysm

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Background: Thrombin-Activatable Fibrinolysis Inhibitor (TAFI) is an inhibitor of fibrinolysis and inflammation. Mice deficient in TAFI

develop larger abdominal aortic aneurysms (AAA) after elastase infusion than controls. The monoclonal antibody (MA-TCK26D6), selectively inhibits plasmin-mediated TAFI activation and TAFI activity on fibrin only.

Aims: The aim of this study was to investigate the effect of clot-based vs. systemic TAFI inhibition on AAA formation *in-vivo*.

Methods: Male, ApoE^{-/-} mice were infused with Angiotensin II (AngII) using subcutaneous mini-osmotic pumps, in combination with either MA-TCK26D6, which has its main effect on TAFI in thrombus, or UK-396082, a direct small molecule inhibitor of the active site of TAFIa. Incidence of AAA rupture was recorded. After 28 days, blood was collected from the IVC, and aortas photographed *in situ*. Aortic size was measured using Image J. Fibrin clot structure in mice receiving AngII was analysed using turbidity-lysis and confocal microscopy.

Results: There were no differences in *ex-vivo* fibrin clot structure in mice treated with AngII compared with saline controls. All mice had normal phenotype, without increases in blood pressure or heart rate. Administration of MA-TCK26D6 and UK396082 led to a decrease in mortality secondary to aortic rupture (from 36.8% to 10.0% and 8.3% respectively). MA-TCK26D6 led to a decrease in AAA incidence (58.3–33.0%), whereas UK396082 resulted in an increase (58.3–81.8%). Where AAA did occur, neither inhibitor resulted in a change in AAA size.

Conclusion: Infusion of AngII did not alter *ex-vivo* fibrin clot structure. Inhibition of plasmin-mediated activation of TAFI *in-vivo* resulted in a decrease in the incidence of AAA likely due to localised fibrinolysis with unaltered inflammatory activity, whereas inhibition of all TAFI activity resulted in an increase in AAA formation likely due to increased inflammatory response.

Disclosure of Interest: None declared.

AS206

Role of DNA methylation in candidate genes regions on tissue plasminogen activator levels

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Background: Tissue Plasminogen Activator (tPA) is a serine protease that mediates the conversion of plasminogen to plasmin, the major enzyme responsible for endogenous fibrinolysis. In some populations, elevated tPA levels are associated with thrombotic outcomes. tPA plasma levels are highly heritable, with estimates as high as 67%. A GWAS identified three genes involved in tPA regulation. Collectively, these genes explain only ~1% of tPA variance. DNA methylation can regulate gene transcription and may account for variation in tPA levels.

Aims: To identify DNA methylation levels, in candidate genes regions, associated with tPA plasma levels.

Methods: In discovery sample of 140 individuals from 5 extended French-Canadian families ascertained on a single proband with venous thromboembolism, a linear mixed regression model adjusted for relatedness and covariates was used, to test for association between DNA methylation levels and tPA plasma levels. DNA methylation sites located within 150 kb of *PLAT*, *STXB5*, and *STX2* gene regions were tested. Sites with FDR < 0.1 were tested for replication in a study sample of 186 venous thromboembolism cases.

Results: A total of 304 sites were tested for association. Significant associations with tPA levels were identified for 5 sites within *PLAT* region, 1 site within *STXBP5* region, and 7 sites within *STX2* region. Associations for 3 sites (2 in *PLAT* and 1 in *STX2*) were replicated in an independent study sample ($P < 0.03$). In *PLAT* region, both sites are located upstream of the gene and in active genomic regulatory regions (transcription factor binding sites). The third site is located within the *STX2* gene, which has been previously shown to be associated with tPA release. Collectively, these methylation sites explain 5.7% of tPA level variation.

Conclusion: Inter-individual variation in DNA methylation levels in *PLAT* and *STX2* genomic regions contribute to the variation in tPA plasma levels, providing new mechanistic insights on tPA regulation.

Disclosure of Interest: None declared.

Protein C and sepsis

AS207

Protein C (APC) pathway functions in inflammatory diseases

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Abstract Body: Activated protein C has been shown to reduce complications in a variety of disease models including sepsis, diabetes, inflammatory bowel disease, kidney and heart reperfusion injury, asthma and stroke. Thrombomodulin is involved not only in protein C activation but in down regulating complement activation. Mutations in thrombomodulin have been identified that eliminate its ability to inhibit complement activation. Patients with these mutations appear to be susceptible to atypical hemolytic uremic syndrome, the onset of which is often linked to some inflammatory stimulus. The endothelial protein C receptor is involved in binding protein C and increasing protein C activation rates. It also binds activated protein C and that complex enhances endothelial barrier function and decreases elaboration of adhesion molecules on endothelium, processes that involve activation of protease activated receptors. EPCR also serves as a binding site for malaria infected red cells. This interaction appears to contribute to stroke in infected children. In many inflammatory diseases, cells release DNA histone complexes. The histones activate toll like receptors 2, 4 and 9 thereby triggering an inflammatory response. Activated protein C can cleave the most active histones and thereby block their pro-inflammatory activity. Thus, the pathway serves many functions in modulating inflammatory diseases.

Disclosure of Interest: None declared.

AS208

Silencing antithrombin and protein C in ApoE knockout mice causes severe coagulopathy but not atherothrombosis

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Background: Atherothrombosis is a major cause of cardiovascular events. Unfortunately, mouse models rarely develop atherothrombosis due to their strong anticoagulation activity and relatively stable atherosclerotic plaques. In models for atherothrombosis often the latter obstacle is overcome by physically inducing plaque rupture. However,

it has not been studied whether reducing anticoagulation activity leads to atherothrombosis in mice.

Aims: Recently, a novel mouse model was developed in which spontaneous venous thrombosis was induced by siRNA targeting of the anticoagulation factors *Serpinc1* (antithrombin) and *Proc* (protein C). We have used this model to study the role of anticoagulation in arterial and venous thrombosis in dyslipidemic and atherosclerotic Apolipoprotein E knockout (ApoE^{-/-}) mice.

Methods: ApoE^{-/-} mice were fed Western Type Diet for 8 weeks, followed by injection with siRNA targeting *Serpinc1*, *Proc*, or both.

Results: Combined knockdown of *Serpinc1* ($-98.7 \pm 0.4\%$) and *Proc* ($-95.4 \pm 0.4\%$) led to severe coagulopathy, including a massive decrease in circulating platelets ($-94.3 \pm 0.8\%$), severe weight loss ($-16.6 \pm 3.2\%$), microscopically visible venous thrombi and subsequent hepatic ischemia within 48 h post injection. Single gene targeting (*Serpinc1*: $-96.0 \pm 1.2\%$, *Proc*: $-90.8 \pm 0.6\%$) caused no macroscopically visible signs of coagulopathy, but circulating platelets were decreased (*Serpinc1*: $-36 \pm 11\%$, *Proc*: $-53 \pm 21\%$), albeit to a lesser extent than when both genes were targeted. Atherothrombosis was not observed upon either single or double gene knockdown.

Conclusion: In conclusion, combined silencing of *Serpinc1* and *Proc* causes severe coagulopathy in ApoE^{-/-} mice. Furthermore, this study shows that atherothrombosis cannot be induced in ApoE^{-/-} by combined or individual silencing of *Serpinc1* and *Proc*. Nonetheless, this model is valuable for future research on the interplay between hyperlipidemia and thrombosis.

Disclosure of Interest: None declared.

AS209

E-WE thrombin activates protein C on the platelet surface and rapidly interrupts experimental thrombus progression in primates

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Background: E-WE thrombin is a protein C activator thrombin analog that is currently in preclinical development for treating acute thrombosis.

Aims: We evaluated whether E-WE thrombin can interrupt thrombus propagation in a baboon vascular graft thrombosis model and explored a mechanism by which E-WE can activate protein C in the absence of an intact endothelium.

Methods: The ability of E-WE thrombin to interrupt thrombus formation was compared with tissue plasminogen activator (tPA) in baboons. Thrombosis was initiated in collagen coated teflon vascular grafts (2 or 4 mm ID) within a chronic arterio/venous shunt. Radio-labeled fibrinogen and platelet deposition were monitored to quantify thrombus growth for up to 90 min. E-WE or tPA were injected IV 15 or 30 min after thrombus initiation. For *in vitro* studies, purified platelets were stimulated with collagen and incubated with E-WE thrombin and protein C to measure generation of activated protein C (APC).

Results: In the 4 mm grafts, tPA (1 mg kg^{-1}) reduced platelet accumulation by 13% at 90 min after thrombus initiation, compared to untreated controls ($n = 6$ each). E-WE thrombin ($1.5\text{--}10 \text{ } \mu\text{g kg}^{-1}$) rapidly and dose-dependently interrupted platelet deposition by 46–71% ($n = 14$) compared with controls. In the 2 mm grafts, all 8/8 control devices occluded within 30 min. E-WE thrombin ($2.5\text{--}10 \text{ } \mu\text{g kg}^{-1}$) interrupted and prevented occlusive thrombus formation, with only 4/12 devices occluding within 60 min. E-WE treatment did not alter bleeding times or volumes. *In vitro*, activated platelets significantly enhanced APC generation by E-WE, which was blocked by an anti-thrombomodulin antibody.

Conclusion: E-WE thrombin effectively interrupts experimental occlusive thrombus formation in primates, likely by platelet-mediated targeting of APC generation to the thrombus surface. E-WE may therefore provide a safe and effective treatment for acute thrombotic cardiovascular emergencies such as heart attack and stroke.

Disclosure of Interest: E. Tucker Shareholder of: Aronora, Inc, Employee of: Aronora, Inc, M. Wallisch Employee of: Aronora, Inc, N. Verbout Employee of: Aronora, Inc, B. Markway Employee of: Aronora, Inc, O. McCarty: None Declared, A. Gruber Shareholder of: Aronora, Inc, Employee of: Aronora, Inc.

AS210

Impact of the endothelial protein C receptor in coagulation, inflammation and hematopoiesis

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Background: The endothelial protein C receptor (EPCR) binds to protein C (PC) and increases the rate of activated protein C (APC) generation by the thrombin-thrombomodulin complex. APC exerts anticoagulant, anti-inflammatory, and cytoprotective effects, which are EPCR-dependent. In addition to the endothelium, EPCR is highly expressed on hematopoietic stem cells (HSC) although the biological role for EPCR on HSCs is unknown.

Aims: In order to study the role of EPCR independent of PC interaction we generated an EPCR point mutation knock-in mouse (EPCR^{R84A/R84A}) which lacks the ability to bind PC/APC. We hypothesize that impaired PC/APC binding to EPCR will result in a procoagulant and pro-inflammatory phenotype, and will modulate hematopoiesis.

Methods: Blood collected from mice after thrombotic or inflammatory challenge was analyzed for markers of coagulation and inflammation. Neutrophil infiltration was assessed by myeloperoxidase activity. Fibrin deposition was assessed by immunohistochemistry. Bone marrow transplant (BMT) experiments were performed on irradiated wild-type (WT) and EPCR^{R84A/R84A}. Spleen and bone marrow cells were collected 6 months post BMT and analyzed by flow cytometry.

Results: EPCR^{R84A/R84A} mice infused with factor Xa generate less APC, and more thrombin-antithrombin complexes, and show increased fibrin deposition in lungs compared to WT controls. EPCR^{R84A/R84A} mice challenged with lipopolysaccharide generate less APC, more IL-6 and show increased neutrophil infiltration in the lungs compared to WT controls. Interestingly, EPCR^{R84A/R84A} mice develop a splenic disorder characterized by splenomegaly and extramedullary hematopoiesis. BMT experiments suggest that the splenomegaly is due to bone marrow failure.

Conclusion: These results show that impaired PC binding to EPCR not only has procoagulant and pro-inflammatory effects, but also impairs bone marrow function, suggesting a role for EPCR in hematopoiesis.

Disclosure of Interest: None declared.

Chronic kidney disease and coagulation

AS211

Chronic kidney disease as a risk factor for thrombotic and bleeding events

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With the increasing worldwide prevalence of chronic kidney disease, it is anticipated that medical complications will rise in parallel. Patients with chronic kidney disease and requiring dialytic therapies are getting older, with more co-morbid medical conditions, interventions and polypharmacy all leading to increases in the risk of venous thromboembolism and bleeding events. In this session we will review the epidemiological risk of venous thromboembolism and bleeding events among those with kidney disease. Risk will be examined across the kidney disease spectrum (chronic kidney disease, dialysis and renal transplantation) and among specific subgroups (the elderly, antithrombotic medications). Lastly mechanisms for the increased risk and an overview for harm reduction will be discussed.

Disclosure of Interest: None declared.

AS212

GRP78 injection ameliorates haemolytic uraemic syndrome (HUS) phenotype in a subAB toxin induced mouse model

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Background: Haemolytic uraemic syndrome (HUS) presents with thrombocytopenia, red cell fragmentation and renal failure. Shiga toxinigenic Escherichia coli (STEC) causes HUS by Shiga toxin elaboration. Recently, Subtilase cytotoxin (SubAB) has also been implicated. SubAB single handedly recapitulates a mouse model of HUS. The A unit is internalised intracellularly and selectively cleaves GRP78, an endoplasmic reticulum chaperone protein. Depletion of GRP78 triggers induction of the unfolded protein response and cell death. Treatment of HUS is supportive with often disastrous consequences, including chronic renal failure and death.

Aims: 1) To see if GRP78 used as a 'decoy molecule' can ameliorate the HUS phenotype in a mouse model. 2) To assess whether a mutant GRP78, D416, which is resistant to cleavage by SubAB, can further ameliorate the HUS phenotype.

Methods: A 5 wk old male BALB/c mice received intraperitoneal (IP) injection of SubAB and either concurrent intravenous (IV) injection of wild type GRP78 (WT GRP78) or GRP78 D416. Mice were sacrificed at 72 h.

Results: SubAB alone caused severe thrombocytopenia in 50% of mice (8/16) at 72 h. IV administration of WT GRP78 (8 µg g⁻¹) or GRP78 D416 (8 µg g⁻¹) concurrently with SubAB (2.5 µg) prevented thrombocytopenia in 100% of the mice (11/11 and 4/4 respectively). Delaying GRP78 administration until 3 h post SubAB injection did not prevent this. Mice lost weight in all groups treated with SubAB, however in the groups treated with concurrent WT GRP78, weight loss at 72 h was less severe. There was no detectable difference in degree of red cell fragmentation or haemoglobin. Renal damage at 72 h was too mild to detect a difference.

Conclusion: Concurrent injection of GRP78 with SubAB resulted in amelioration of HUS phenotype, manifest as preservation of spleen size and less weight loss. Exogenous GRP78 acts as a decoy for SubAB, but given SubAB depletes constitutive intracellular GRP78, the improvement was minimal.

Disclosure of Interest: None declared.

AS213

Tumor necrosis factor suppresses the glomerular endothelial cell surface protein CD141 (thrombomodulin) and stimulates expression of C3 and factor B: implications for renal injury and thrombosis in the atypical hemolytic uremic syndrome

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Background: Alternative complement pathway (AP) dysregulation and kidney injury characterize atypical Hemolytic Uremic Syndrome (aHUS). CD141 (thrombomodulin) is an endothelial cell (EC) surface protein that prevents complement-mediated injury and serves as a natural anticoagulant. Differences in the production of complement regulatory proteins and AP components by glomerular microvascular endothelial cells (GMVECs), compared to human umbilical vein endothelial cells (HUVECs), may explain the renal injury of aHUS. These differences may be augmented during inflammation.

Aims: Quantify complement regulatory protein and AP component gene expression in GMVECs and HUVECs, and quantify surface complement regulatory proteins on both cell types, with and without tumor necrosis factor (TNF) and interleukin 1-beta (IL1- β) stimulation.

Methods: HUVEC and GMVEC RNA for CD55, CD46, CD59, CD141 and AP components were analyzed by real-time PCR. The fold changes in gene expression were calculated in control cells and cells exposed to TNF or IL1- β for 48 h. HUVECs and GMVECs stimulated for 48 h with TNF or IL1- β were also labeled with FITC- or PE-conjugated antibodies to CD55, CD46, CD141 and CD59 or with isotype controls, and analyzed by flow cytometry.

Results: Thrombomodulin gene (*THBD*) expression was 2-fold higher and CD141 surface presence was 7-fold higher in GMVECs as compared to HUVECs. Gene expression of C3 increased 32-fold in TNF-incubated HUVECs and 153-fold in TNF-incubated GMVECs. Gene expression of *CFB* increased 16-fold in TNF incubated HUVECs and 59-fold in TNF-incubated GMVECs.

Conclusion: *THBD* gene expression in GMVECs is extensively down-regulated by TNF, decreasing CD141 presence and leading to renal microvascular vulnerability to complement-mediated injury and micro-thrombi formation. AP components C3 and CFB are extremely up-regulated in GMVECs by TNF. Augmented AP activity plus decreased GMVEC protection explain the susceptibility of the kidney to injury during inflammation, and in aHUS.

Disclosure of Interest: None declared.

AS214

Long-term tinzaparin versus warfarin for treatment of venous thromboembolism (VTE) in cancer patients – analysis of renal impairment (RI) in the catch study

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Background: Cancer patients receiving anticoagulation for VTE have elevated risks for recurrent VTE (rVTE) and bleeding. RI also increases rVTE and bleeding risk.

Aims: To assess the impact of RI on rVTE or clinically relevant bleeding (CRB; major or clinically relevant non-major bleeding) in cancer patients receiving anticoagulation.

Methods: CATCH, a Phase III PROBE trial (NCT01130025), compared tinzaparin (T) 175 IU kg⁻¹ once daily for 6 months vs. initial T 175 IU kg⁻¹ once daily for 5–10 days and warfarin (W) for 6 months in patients with active cancer and acute symptomatic VTE. Dose of T was not adjusted for renal function. Risk of rVTE and CRB was compared in patients with CrCl ≥ 60 mL min⁻¹ vs. CrCl < 60 mL min⁻¹. Patients were followed up to 6 months or death for rVTE and up to 24 h after last dose of study drug for CRB.

Results: A total of 862 subjects had a central lab CrCl value at randomization; 733 had CrCl ≥ 60 mL min⁻¹ and 129 had CrCl < 60 mL min⁻¹. Mean age was 59 years; 41% were male. rVTE occurred in 8% of patients with CrCl ≥ 60 mL min⁻¹ vs. 14% of patients with CrCl < 60 mL min⁻¹ (relative risk [RR] 0.57; 95% CI 0.35–0.93). Corresponding rates were 6% (22/355) vs. 13% (9/67; RR 0.46; 95% CI 0.22–0.96) among those treated with T and 10% (36/378) vs. 15% (9/62; RR 0.66; 95% CI 0.33–1.29) among those on W. There were no statistically significant differences in rVTE between T and W within each renal group.

CRB occurred in 15% of patients with CrCl ≥ 60 mL min⁻¹ vs. 20% of patients with CrCl < 60 mL min⁻¹ (RR 0.75; 95% CI 0.51–1.10). Corresponding rates were 13% (46/355) vs. 16% (11/67; RR 0.79; 95% CI 0.43–1.44) among those on T and 17% (65/378) vs. 24% (15/62; RR 0.71; 95% CI 0.43–1.16) among those on W. There were no statistically significant differences in CRB between T and W within each renal group.

Conclusion: RI leads to a significant increase in the risk of rVTE but not CRB in cancer patients on anticoagulation for VTE. Long-term T therapy in RI patients did not increase CRB compared with W.

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Endothelial cell-VTE models

AS215

The role of GAS6 in the pathophysiology of venous thrombosis

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Gas6 is a vitamin K dependent protein that is homologous to protein S but has no role in thrombin generation and fibrin formation. Rather, it is a soluble ligand for the TAM receptor tyrosine kinases with protean roles in several different cellular systems. Gas6 has a role in both venous and arterial thrombosis as demonstrated in gas6 null mice. These mice, lacking gas6, were protected from both arterial thrombosis and lethal venous thromboembolism through a defect in platelet signalling.

To investigate other possible contributions to thrombus formation by Gas6, we employed a bone marrow transplantation strategy to generate mice with selective ablations of Gas6 in the vascular or hematopoietic compartments. This approach successfully allowed us to observe a contribution by vascular Gas6 to thrombus formation, through the promotion of tissue factor upregulation in endothelial cells.

Based on recent evidence that (i) Gas6 regulates the expression of tissue factor during venous thrombosis and (ii) cancer promotes a pro-coagulant milieu, we hypothesized that Gas6 may be involved in cancer-induced coagulopathy. We demonstrate that gas6 null mice were protected from cancer-induced coagulopathy in a murine model of Cancer-associated thrombosis. Whole genome microarray analysis of differential gene expression in WT and Gas6^{-/-} endothelial cells co-cultured with M27 murine lung carcinoma cells reveal that Gas6 increased prostaglandin E synthase expression in endothelial cells, resulting in augmented PGE₂ production. PGE₂ activates platelets after binding to its receptor, EP3. *In vivo*, EP3 receptor antagonism reversed the effect of cancer-induced thrombosis in WT mice. These results show that Gas6, through upregulation of PGE₂, contributes to cancer-induced venous thrombosis.

In summary, the above findings suggest a potential novel mechanism for thrombus formation and aid in the understanding of the pathophysiology of venous thrombosis.

Disclosure of Interest: None declared.

AS216

Correlation between deep vein thrombosis and pulmonary embolism using an *in vivo* mouse model of venous thromboembolism: effect of thrombin inhibition

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Background: Whether a patient presents with deep vein thrombosis (DVT) or pulmonary embolism (PE) varies based on clinical factors. Patients with factor V Leiden typically present with DVT while cancer patients present with PE. The role of DVT stability in the progression of DVT to PE is not known. Clinical trial data suggest an increase in thrombotic events when patients were treated acutely with a direct thrombin inhibitor (DTI) compared to low-molecular-weight-heparin (LMWH).

Aims: To develop a model to assess thrombus stability and its link to PE burden, and whether DTIs, as opposed to LMWH, alters this correlation.

Methods: Platelets were fluorescently labelled using CD41 fab fragments conjugated to an Alexa Fluor 488. The femoral vein of C57BL/6 female mice was subjected to ferric chloride injury to initiate thrombus formation. Treatment with saline, dalteparin (0.2 IU g⁻¹ mouse body weight (mbw)), or dabigatran (33 mg g⁻¹ mbw), was administered 12 min after thrombus formation. Intravital videomicroscopy recorded embolic events (EEs) leaving the thrombus, and thrombus sizes (TSs) every 10 min for 2 h. Lungs were harvested, sectioned and stained for the presence of PE.

Results: Total and large EEs were highest after dabigatran treatment compared to saline or dalteparin. Between the 1st and 2nd hour after treatment, dabigatran-treated mice showed the highest increase in EEs over time. Variations in amounts of EEs were not attributed to variations in TSs since TSs was similar between the groups. The number of emboli per lung slice was higher in dabigatran-treated mice. Large EEs correlated positively with the number of emboli per lung slice independent of treatment.

Conclusion: We have developed a mouse model of venous thromboembolism that can quantify emboli and PE burden. Consistent with clinical data, dabigatran, a DTI, was shown to acutely decrease thrombus stability and increase PE burden compared to LMWH or saline.

Disclosure of Interest: None declared.

AS217

Inhibition of platelet function with the novel 12-LOX inhibitor ML355 impairs thrombus growth, stability and vessel occlusion *in vivo*

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Background: Platelet activation is a key step in hemostasis; however excessive level of reactivity can lead to occlusive thrombi. While current treatments limit platelet function, they often result in an increased risk of bleeding. 12-lipoxygenase (12-LOX), an oxygenase highly expressed in the platelet, has been demonstrated by our lab and others to regulate PAR4 and GPVI-mediated platelet reactivity. The ability to target 12-LOX *in vivo* has not been established to date.

Aims: To determine how 12-LOX regulates thrombus formation *in vivo*. 12-LOX deficient (12-LOX^{-/-}) mice or wild-type (WT) mice treated with or without with the 12-LOX inhibitor, ML355, were assessed for effects on platelet activation and thrombus formation.

Methods: *In vivo* thrombus formation was studied in 12-LOX^{-/-}, WT mice, and mice treated with ML355 using intravital microscopy using the FeCl₃ injury model. Additionally, whole blood was tested *ex vivo* following incubation with ML355 for platelet adhesion in real time.

Results: Platelet aggregation and thrombus growth was impaired in response to FeCl₃ injury in 12-LOX^{-/-} mice in the mesenteric or carotid artery thrombosis model. Thrombi in 12-LOX^{-/-} mice were unstable and frequently form emboli which resulted in impaired vessel occlusion or re-opening. Additionally, thrombus formation and vessel occlusion was impaired in ML355 treated WT mice. In the *ex vivo* flow chamber assay, platelet adhesion and thrombus formation at high shear was attenuated in both mouse and human blood after incubation with ML355.

Conclusion: Thrombus growth, stability, and vessel occlusion is impaired in mice deficient for 12-LOX. The highly selective 12-LOX inhibitor ML355 attenuates thrombus formation and prevents vessel occlusion *in vivo*. Further, platelet function at high shear in *ex vivo* conditions in both mice and human was attenuated in the presence of ML355. Our data strongly indicates that inhibition of platelet 12-LOX may represent a new target for anti-platelet therapeutics.

Disclosure of Interest: None declared.

AS218

Tissue factor pathway inhibitor (TFPI): an endogenous substrate for factor VII activating protease (FSAP) *in vivo*

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Background: Factor VII activating protease (FSAP) is a serine protease in plasma. Genetic epidemiological studies have implied a role for FSAP in thrombosis, carotid stenosis and stroke.

Aims: To date, no *in vivo* evidence is available to support these claims. In our present study, we have used FSAP^{-/-} mice to define its role in thrombosis and haemostasis *in vivo* and to characterize the molecular mechanisms involved.

Methods: Wild type (WT) and littermate FSAP^{-/-} mice were compared in various *in vivo* and *ex-vivo* model systems. To study the arterial system, we performed FeCl₃-induced carotid artery and mesenteric arteriole models. We also performed pulmonary embolism model to study the function of FSAP in venous system. To evaluate the haemostasis tail-bleeding assay was performed. All the downstream *ex-vivo* analysis (coagulation factor, platelet function, TFPI, etc.) was performed using the samples from above models.

Results: FeCl₃-induced arterial thrombosis revealed that the occlusion time was significantly increased in FSAP^{-/-} mice ($P < 0.01$) and that some FSAP^{-/-} mice did not occlude at all. Lethal pulmonary thromboembolism induced by collagen/epinephrine infusion was delayed in FSAP^{-/-} mice ($P < 0.01$). Although no spontaneous bleeding was evident, in the tail-bleeding assay, a re-bleeding pattern in FSAP^{-/-} mice was observed. Thus, a lack of endogenous FSAP impaired the formation of stable, occlusive thrombi. To explain these observations at a mechanistic level we then determined how haemostasis factors and putative FSAP substrates were altered in FSAP^{-/-} mice. Similarly, when reconstitute the FSAP, the phenotype of FSAP^{-/-} mice was reversed and significantly reduced the TFPI level. Interestingly, we could not find any evidence for altered activation of factor VII in FSAP^{-/-} mice.

Conclusion: Thus, we conclude TFPI is likely to be a substrate for FSAP, rather than FVIIa, which accounts for its effects in thrombosis and haemostasis.

Disclosure of Interest: None declared.

AS219

Intrinsic and extrinsic coagulation pathways both contribute to ligation-induced deep vein thrombosis in rats

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Background: Previously we showed rapid tissue factor expression after inferior vena cava (IVC) ligation-induce deep vein thrombosis in rats implicating the extrinsic pathway in this process. However, it is unknown whether the intrinsic pathway also contributes because of activation by nucleic acids.

Aims: To determine the relative contribution of the extrinsic and intrinsic pathways to ligation-induced DVT in rats, and to investigate the role of DNA or RNA in this process.

Methods: Antisense oligonucleotides (ASOs) were used to lower the levels of factor (f) VII or fXII prior to ligation, and some rats given the fVII-directed ASO received concomitant corn trypsin inhibitor (CTI) at the time of ligation to inhibit fXIIa. The effect of CTI alone also was examined. Intravenous DNase or RNase was administered prior to ligation to determine their role in DVT. Thrombi were harvested and subjected to immunohistochemical analysis.

Results: Compared with saline, fVII- and fXII-directed ASOs reduced fVII and fXII activities to $0.1 \pm 0.2\%$ and $3 \pm 1\%$, respectively, and reduced IVC thrombus mass to $25 \pm 15\%$ and $20 \pm 13\%$, respectively. Whereas CTI alone reduced thrombus mass to $35 \pm 27\%$ relative to saline, CTI administration to rats given the fVII ASO reduced thrombus mass to $3 \pm 7\%$; a value significantly ($P < 0.05$) lower than that with fVII ASO or CTI alone. DNA, but not RNA, was seen in thrombi, and prior administration of DNase, but not RNase, reduced thrombus mass to $18 \pm 21\%$ relative to the saline control. DNA particles were abundant throughout the thrombi, but neutrophil extracellular traps were rare. Leukocytes in the thrombi stained for DNA, cleaved caspase-3, and hypoxia inducible factor-1a.

Conclusion: The intrinsic and extrinsic pathways play independent but cooperative roles in ligation-induced DVT in rats. DNA contributes to thrombosis in this model and stasis and hypoxia may induce cell apoptosis, netosis, and DNA release.

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Inhibitors – clinical

AS220

Factor VIII inhibitors in patients with non-severe hemophilia

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Although neutralizing antibodies (inhibitors) that bind factor (F) VIII most commonly occur in the setting of severe hemophilia A, they can also be a devastating complication of FVIII replacement therapy in 5.3% of patients non-severe hemophilia A. When compared with severe hemophilia A, they tend to occur at an older age (median age of 46 years vs. 15 months) and after greater FVIII exposure (28 vs. 14 exposure days). Furthermore, the risk increases up to 100 exposure days. Over the past 5 years, a greater understanding of risk factors for inhibitor development in this population has developed. Important risk factors include intensive exposure to FVIII, particularly in adult patients, and specific F8 mutations such as Arg593Cys, Trp2229Cys, and Arg2159Cys mutation. Other risk factors that have been postulated but are less well understood include surgery as the indication for intensive replacement therapy and the method of delivery of intensive FVIII replacement. Once inhibitors develop in persons with non-severe hemophilia A, they often cross-react with native FVIII leading to a reduction in circulating FVIII activity and marked change in bleeding phenotype. The bleeding phenotype more closely corresponds to that seen with acquired hemophilia A with muscle and soft-tissue bleeding rather than spontaneous joint bleeding. For treatment of bleeding episodes a variety of approaches have been proposed. The best approach to inhibitor eradication remains a matter of debate. Immune tolerance induction (ITI) as is carried out in patients with severe hemophilia is typically not useful or necessary likely because of

the tolerogenic effect of continued circulating endogenous FVIII. Rituximab continues to be considered a first line approach by many, though its benefit over observation alone is unclear. Once the inhibitor has resolved, recurrence rates are unknown, though re-introduction of FVIII replacement therapy has been reported even in the absence of prior ITI therapy.

Disclosure of Interest: None declared.

AS221

State-of-the-art analysis of FVIII-binding antibodies reveals IGA as a prognostic biomarker for poor treatment outcomes in acquired hemophilia A

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Background: Acquired hemophilia A (AHA) is a serious autoimmune disorder caused by neutralizing autoantibodies against factor VIII (FVIII). Remission can be achieved by immunosuppressive therapy and time to remission ranges from a few days to several months. The GTH-AH 01/2010 study included patients treated according to a uniform protocol and established that baseline FVIII and FVIII inhibitor titers are predictors of the time to partial remission (PR) or complete remission (CR) (Tiede et al. Blood 2015).

Aims: To assess the potential prognostic value of state-of-the-art analysis of FVIII-binding antibodies.

Methods: Antibody titers and apparent affinities of FVIII-binding antibodies, differentiated for IgM, IgG 1–4 and IgA, at time of diagnosis from 81 patients were analyzed as described by Whelan et al. (Blood 2013) and Hofbauer et al. (Blood 2015). Univariate and multivariate Cox regression models were used to determine hazard ratios (HR) with 95% confidence intervals (CI) for the prognostic impact of these markers on time to PR, CR, and overall survival (OS), and were adjusted for confounding factors.

Results: Patients positive for FVIII-binding IgA (46%) at time of diagnosis achieved CR less often (HR 0.35, CI 0.18–0.68) and had a higher mortality (HR 2.6, CI 1.1–6.2). Effects on PR were of borderline significance, as were any effects of the presence or absence of other antibody subclasses. Furthermore, patients with IgA titers > 1:80 (median) had a worse prognosis than those with lower titers. Of note, IgA titers did not correlate with FVIII inhibitor titers, whereas IgG subclass titers did. Apparent affinities of FVIII-binding antibodies did not appear to have an impact on outcomes except for effects of marginal significance on PR.

Conclusion: FVIII-binding IgA autoantibodies at time of diagnosis are a prognostic biomarker associated with poor prognosis in AHA, as positive patients achieved CR less often and showed decreased OS in an IgA titer-dependent manner.

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AS222

Predictors of success of immune tolerance induction in hemophilia A patients with high-responding inhibitors. A prognostic score from the Italian profit registry

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Background: Predictors of success of immune tolerance induction (ITI) in patients with hemophilia A and high-responding inhibitors are still debated.

Aims: To analyze predictors of outcome from 137 first ITI courses in the Italian retrospective-prospective Registry.

Methods: ITI outcome was centrally reviewed (success: undetectable inhibitor, *in vivo* recovery > 66%, FVIII half-life > 6 h; partial success: inhibitor titre < 5 BU mL⁻¹ and/or abnormal pharmacokinetics, clinical response to FVIII treatment). A multivariate analysis of predictors was adjusted for patient- and treatment-related potential confounders.

Results: ITI was started at -median (range)- 4.8 years (0.3–58.5), with pre-ITI inhibitor titer of 4.5 BU mL⁻¹ (< 0.5–200), 19 months (0–332) after inhibitor diagnosis. Historical inhibitor peak titer was 64 BU mL⁻¹ (6–920). VWF-containing products were used in 24% of cases and recombinant FVIII in the remaining (≥ 100 IU kg⁻¹ per d in 33% and 75% and daily regimens in 48% and 83%, respectively, *P* < 0.01). Inhibitor peak titre during ITI was 64 BU mL⁻¹ (5–16,384). Success was achieved by 70 patients (51.1%), partial success by 21 (15.3%). Success rates were similar (53% vs. 49%) in 'good-risk' and 'poor-risk' patients (Hay & Di Michele, Blood 2012). Pre-ITI inhibitor titre (< 5 BU mL⁻¹, adjusted OR [95% CI] 6.5 [1.8–11.2], *P* < 0.001), historical peak titre (< 200 BU mL⁻¹, 2.6 [1.2–6.5], *P* < 0.05) and peak titre during ITI (< 100 BU mL⁻¹, 8.2 [3.1–22.8], *P* < 0.001) were significant predictors of success, as well F8 mutation class (*non-null* vs. *null* genotypes 79% vs. 45%, 11.1 [2.76–44.7], *P* = 0.001). A prognostic score, assigning 1 point in the absence of each favourable predictor, was inversely related (*P* < 0.001) with success rate (0: 91%; 1: 79%; 2: 43%; 3: 24%; 4: 8%) and time to achieve success (median, mo, 0: 7; 1: 11; 2: 16; 3: 23).

Conclusion: These data suggest that definitions of 'good-risk' and 'poor-risk' patients should be revisited, and contribute to identify a prognostic profile useful for optimizing clinical choices in ITI management.

Disclosure of Interest: None declared.

AS223

Risk for inhibitor development in severe hemophilia A pups is not associated with class of FVIII concentrates nor by high von-Willebrand factor or by type of viral inactivation procedure

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Background: Most studies have demonstrated a higher risk of inhibitor development for class recombinant vs. class plasma-derived concentrates. Debate is ongoing what the impact is of high von-Willebrand factor (vWF) containing plasma-derived concentrates and whether different viral inactivation procedures might be associated with a different risk for inhibitors.

Aims: To investigate the association of plasma-derived vs. recombinant FVIII concentrates, vWF content and type of viral inactivation procedure on inhibitor development in previously untreated patients (PUPs) with severe hemophilia A.

Methods: A total of 883 PUPs with severe hemophilia A (FVIII < 1%) born in 1990–2009 were included. Patients were followed until 50 exposure days. Risk of inhibitor development was assessed for classes plasma-derived vs. recombinant concentrates. Furthermore the impact of high vWF content and the method of viral inactivation for plasma-derived concentrates on inhibitor development were investigated. Cox regression with time-varying covariates was used as analysis technique; hazard ratios were adjusted for known genetic and treatment-related variables.

Results: The risk of all and high titer inhibitor development in plasma-derived ($n = 225$) and recombinant concentrates ($n = 658$) was similar with adjusted hazard ratios (aHR) of 0.78 (95% CI 0.56–1.07) for all and 0.80 (0.55 – 1.14) for high titer inhibitors. Low vWF containing concentrates ($n = 41$) had aHR of 0.77 (0.34 – 1.76) for all inhibitors and 0.85 (0.34 – 2.12) for high titer inhibitor development compared with high vWF concentrates ($n = 184$). Lastly, no effect of different viral inactivation methods on inhibitor development was demonstrated.

Conclusion: The risk of classes plasma-derived and recombinant concentrates for inhibitor development was similar. Low vWF containing concentrates had the same risk comparing with high vWF containing concentrates. No effect of different viral inactivation methods on inhibitor development was observed.

Disclosure of Interest: None declared.

AS224

Mutation analysis of Swedish hemophilia B families – high frequency of unique mutations

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Background: Hemophilia B (HB) is caused by a heterogeneous spectrum of mutations. Characterization of the mutations is important in genetic counseling, prenatal diagnosis and as a determinant to predict risk of inhibitor development.

Aims: To study the spectrum of mutations, the true frequency of unique recurrent mutations and the genotype–phenotype association in the Swedish HB population.

Methods: The study included, due to centralized DNA diagnostics, the whole registered Swedish HB population (113 families: 47 severe, 22 moderate and 44 mild), each represented by one patient in the study. Characterization of mutation was performed by conventional sequencing of all exons and haplotyping by single nucleotide polymorphisms and microsatellites. The study was approved by the regional ethical review board and informed consent was obtained from participating individuals.

Results: A mutation was found in every family: eight had large deletions, three small deletions (< 10 basepair [bp]) and 102 single bp substitutions (69 missense, 26 nonsense, four splice site and three promotor). Eleven mutations had not previously been reported. The 113 families had 66 different mutations. Sixteen mutations (one total gene deletion, 14 single bp and one acceptor splice site) were present in more than one family, accounting for mutations in 60 of the 113 families. In a subsample of 47/60 of these families haplotyping revealed that 51% (24/47) were identical by descent (*i.e.* related) and 49% (23/47) were recurrent mutations. Inhibitors developed in 9/47 (19%) patients with severe HB.

Conclusion: We found eleven unreported mutations in the Swedish HB population. Haplotyping of families with identical mutations revealed the frequency of true unique mutations, based on a subsample, to be at least 79%. The high frequency of inhibitors is probably caused by many 'null' mutations.

Disclosure of Interest: None declared.

Stroke

AS226

Pre-event CHA₂DS₂-VASc score and severity of acute stroke in patients with atrial fibrillation: findings from the RAF study

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Background: Current guidelines recommend CHA₂DS₂-VASc score to assess the risk of stroke in patients with atrial fibrillation (AF). The association between pre-event CHA₂DS₂-VASc score and the severity of acute stroke has never been defined.

Aims: We investigated the association between CHA₂DS₂-VASc score and the severity of acute stroke in a prospective multicentre study which enrolled consecutive patients with acute stroke and AF (RAF study).

Methods: Severity of stroke was evaluated on admission by the National Institute of Health Stroke Scale (NIHSS) score, that was considered both as a continuous and dichotomized variable (severe stroke = NIHSS > 10). Correlations between severity of stroke and pre-event CHA₂DS₂-VASc was evaluated using multiple logistic regression after adjustment for other risk factors.

Results: Of the consecutive patients enrolled in the study, 598 patients had an admission NIHSS score greater than 10 and 399 patients lower than 10. The mean NIHSS scores for CHA₂DS₂-VASc score of 0–8/9 were: 5.18, 8.30, 8.31, 8.86, 9.12, 9.67, 9.70, 10.30, 12.09, respectively. A linear correlation was found between severity of stroke and CHA₂DS₂-VASc score ($r^2 = 0.010$, $P = 0.001$). On multivariate analysis, CHA₂DS₂-VASc score correlated with the severity of stroke (OR 1.084, $P = 0.041$, for each point increase).

Conclusion: In patients with AF, CHA₂DS₂-VASc score is a predictor of severity of stroke in addition to be a predictor of risk of stroke. This observation increases the value of the assessment of CHA₂DS₂-VASc score when considering the use of anticoagulant treatment.

Disclosure of Interest: None declared.

AS227

Early recurrence and cerebral bleeding in patients with acute ischemic stroke and atrial fibrillation: effect of anticoagulation and its timing. The (RAF) study

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Background: Anticoagulation timing in acute cardioembolic stroke remains controversial.

Aims: In a prospective cohort of patients with acute stroke and atrial fibrillation (AF), we evaluated: 1) the risk of recurrent ischemic event and severe bleeding; 2) the risk factors for recurrence and bleeding; 3) the risk of recurrence and bleeding associated with anticoagulant therapy and its starting time after the acute stroke.

Methods: The primary outcome of this multicenter study was the composite of stroke, transient ischemic attack (TIA), symptomatic systemic embolism, symptomatic cerebral bleeding and major extra-cranial bleeding within 90 days from acute stroke.

Results: Of the 1029 patients enrolled, 123 had 128 events (12.6%): 77 (7.6%) ischemic stroke or TIA or systemic embolism, 37 (3.6%) symptomatic cerebral bleeding and 14 (1.4%) major extra-cranial bleeding. High CHA₂DS₂-VASc score, high NIHSS, large ischemic lesion and type of anticoagulant were predictive factors for primary study outcome. Patients treated with oral anticoagulants alone had a better prognosis compared to those treated with low molecular weight heparins (LMWHs) alone or followed by oral anticoagulants. At adjusted Cox regression analysis, initiating anticoagulants 4 to 14 days from stroke onset was associated with a significant reduction in primary study outcome, compared to initiating treatment before 4 or after 14 days: Hazard Ratio 0.53 (95% CI 0.30–0.93).

Conclusion: Acute stroke in AF patients is associated with high rates of ischemic recurrence and major bleeding at 90 days. Anticoagulant treatment administered between 4 and 14 days from the acute event was associated with improved functional outcome.

Disclosure of Interest: None declared.

AS228

Magnesium transporter MagT1 plays a critical role in thrombo-inflammatory diseases and hemostasis in mice

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Background: Altered Mg²⁺ homeostasis has been described in many cardiovascular diseases, especially in patients with coronary heart disease, arrhythmias and stroke. Elevated Mg²⁺ concentrations in the blood or inside of the cells inhibit aggregation responses and thrombus formation *in vivo*. Although Mg²⁺ influx has been described in platelets, the molecular composition of the Mg²⁺ transport system in general remains elusive. Genetic ablation of magnesium transporter 1 (MagT1) has been shown to reduce the cytoplasmic Mg²⁺ concentration in lymphocytes and results in X-MEN syndrome in males.

Aims: We aim to elucidate the role of MagT1 in thrombo-inflammatory diseases.

Methods: MagT1 knockout mice (MagT1^{-/-}) were analyzed using a wide range of *in vitro* and *in vivo* assays.

Results: Deletion of MagT1 function in platelets resulted in decreased levels of free Mg²⁺ in the cytoplasm. Upon platelet activation, enhanced ATP release, increased Ca²⁺ responses, and slightly elevated integrin activation and degranulation were observed in MagT1^{-/-} plate-

lets. Consequently, MagT1^{-/-} mice displayed faster thrombotic occlusion of FeCl₃-injured carotid and mesenteric arteries. Additionally, reduced bleeding times and enhanced clot retraction were observed in the blood of MagT1^{-/-} mice. In the tMCAO model of ischemic stroke, MagT1^{-/-} mice displayed increased cerebral infarct volumes 24 h after induction of a 30 min ischemia. Using bone marrow chimeric mice, we demonstrated that blood cells, including platelets are responsible for the increased susceptibility of t MagT1^{-/-} mice to focal cerebral ischemia.

Conclusion: These results indicate that altered Mg²⁺ transport through MagT1 in males may be a risk factor for thrombo-inflammatory diseases such as stroke.

Disclosure of Interest: None declared.

Atherosclerosis, inflammation and cancer

AS229

Receptors for plasminogen activators: role in inflammation, atherosclerosis, and cancer

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Tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) are the principal mammalian activators of the fibrinolysis system. By activating plasminogen or by directly cleaving alternate substrates, tPA and uPA have been implicated in the response to injury and tissue remodeling. In addition to fibrin and fibrinogen, targets for plasmin and the plasminogen activators include extracellular matrix proteins, membrane-anchored proteins, and growth factors. tPA and uPA are both multidomain gene products. Binding sites have been identified in the structure of uPA and tPA for receptors with cell-signaling activity. These binding sites are distinct from the serine protease domains. Major receptors for the plasminogen activators include but are not limited to the uPA receptor, uPAR, and the tPA receptors, Annexin A2 and LDL Receptor-related Protein-1 (LRP1). This presentation focuses on uPAR and LRP1. Because each of these receptors responds to proteases that are active at the cell surface and in surrounding tissues, LRP1 and uPAR may serve as 'radar systems' for the cell, facilitating responses to changes in the cellular microenvironment. Robust cell-signaling responses are generated downstream of uPAR and LRP1. These responses control cell survival, cell migration, gene expression, differentiation, and in cancer, stem cell-like properties. Emerging questions regarding the function of uPAR include its role in the development of resistance to targeted anticancer drugs. LRP1, which is expressed by numerous cell types including macrophages, may play a major role controlling innate immunity. Understanding the potential to target plasminogen activator receptors for drug discovery is an exciting question.

Disclosure of Interest: None declared.

AS230

CM-352, a new matrix metalloproteinase inhibitor, is a potent and safe antifibrinolytic agent for the prevention and treatment of hemorrhage

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Background: New therapeutic strategies are required for the prevention of hemorrhaging and blood transfusions, associated with increased

morbidity and mortality in different clinical settings, such as surgery, trauma and brain haemorrhage. Tissue damage and hyperfibrinolysis constitute important contributors to major bleeding events. It has been challenging to discover potent and safe agents that improve upon currently available antifibrinolytics (e.g., tranexamic acid/TXA). Matrix metalloproteinases (MMP) participate in thrombus dissolution through direct fibrin targeting or by enhancing tissue plasminogen activator-induced fibrinolysis.

Aims: MMPs inhibition might constitute a new, effective and safe therapeutic strategy for the prevention of bleeding. Therefore, we designed and synthesized a novel series of optimized inhibitors.

Methods: Following initial biochemical profiling (MMPs activity), prioritized molecules underwent a phenotypic screening process consisting of functional thromboelastometry assays (ROTEM) *in vitro* and bleeding tests *in vivo* (tail bleeding and hepatectomy) in mice.

Results: We have identified a lead compound, CM-352 as an effective and safe small molecule with antihemorrhagic properties. CM-352 (1.4 h half life), inhibits fibrinolysis in human whole blood functional assays and is significantly more effective than TXA in the tail-bleeding model ($P < 0.001$). Moreover, CM-352 reduces blood loss during liver hepatectomy (25% reduction, $P < 0.05$), while TXA and aprotinin had no effect. CM-352 displays optimal pharmacokinetic and safety profiles with no evidence of thrombosis or hemostatic impairment.

Conclusion: We provide evidence that fibrinolysis can be inhibited by targeting MMP and this novel mechanism of action defines a new class of antihemorrhagic agents. Furthermore, CM-352 represents a promising clinical candidate for the acute treatment of bleeding

Disclosure of Interest: None declared.

AS231

Inhibition of complement C3 and fibrinogen interaction: a potential novel therapeutic target to reduce fibrin-related thrombosis risk

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Background: Impaired fibrin clot lysis is associated with increased cardiovascular risk. Complement C3 incorporation into fibrin networks compromises fibrinolysis and therefore modulation of C3-fibrinogen interactions may have therapeutic implications.

Aims: Develop new strategies to improve the fibrinolytic process by interfering with fibrinogen-C3 binding.

Methods: To modulate C3 interaction with fibrinogen, we used a novel technique by screening fibrinogen with a phage display library of 30 billion random, conformational constrained 9 amino-acid (AA) loops in a scaffold protein termed Adhiron. The effect of high affinity fibrinogen binding Adhiron, released by the addition of excess C3, on fibrin clot lysis was assessed in turbidimetric assays. Fibrinogen-C3 interactions were further studied by peptide microarray techniques and molecular modelling.

Results: Following multiple rounds of panning, 48 high affinity fibrinogen binding Adhiron, released by C3, were sequenced, revealing 10 different Adhiron, one of which had sequence homology with C3 (Adhiron A6). In a purified system and in the absence of C3, Adhiron A6 failed to modulate fibrin clot lysis time (mean $\pm$ SEM time to 50% clot lysis was 644 ± 13 and 620 ± 14 s, without and with A6, respectively; $P > 0.1$). However, A6 abolished C3-induced prolongation of clot lysis, reducing lysis time from 728 ± 25 to 632 ± 24 s ($P = 0.01$). The peptide microarray screening of C3 identified two peptide motifs within the β -chain of fibrinogen (residues 424–433 and 435–445 AA)

that bound to C3. Molecular modelling, using PepSite2, predicted Adhiron A6 binding to similar areas on the β -chain of fibrinogen.

Conclusion: Using a novel phage display system, we isolated one Adhiron that shared sequence homology with C3 and abolished C3-induced prolongation of fibrin clot lysis, possibly through interference with C3 interaction with the β chain of fibrinogen. This technique offers the potential to identify new therapeutic targets to improve fibrinolytic efficiency and reduce vascular risk.

Disclosure of Interest: None declared.

AS232

Modulation of recombinant antigenic constructs containing multi-epitopes towards effective reduction of atherosclerotic lesion in B6;129S-LDLR(tm1Her) Apob(tm2Sgy)/J mice

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Background: Atherosclerosis is increasingly recognized as a complex chronic inflammatory disease. Many more studies have extended vaccination against atherosclerosis by using epitopes from self-antigens or beyond and demonstrated that vaccination with antigens or derivatives could reduce the extent of the lesions in atherosclerosis-prone mice.

Aims: To investigate whether AHHC (Apolipoprotein B 100^{688–707} + human heat shock protein [hHSP] 60^{303–312} + hHSP60^{153–163} + Chlamydia pneumoniae protein derived peptide [C]) can be modulated towards increased lesion reduction in mice by creating other two derivatives with a sequential epitope-substitution named RHHC in which A was replaced by an 'R' (complement component 5a receptor^{1–31}) and RPHC with a further 'H' (hHSP60^{303–312}) conversion into 'P' (protease-activated receptor-1^{42–55}) in mice.

Methods: Antigenic epitopes were incorporated into a dendroaspilin scaffold. Immunization of B6;129S-Ldlr^{tm1Her} Apob^{tm2Sgy}/J mice with three constructs. Two weeks after the first immunization, mice were placed on a high-fat diet for 10 weeks.

Results: Immunization of B6;129S-Ldlr^{tm1Her} Apob^{tm2Sgy}/J mice with three constructs elicited production of high levels of antibodies against each epitope (apart from hHSP60^{153–163} and P which induced a low antibody response). Histological analyses demonstrated that the mice immunized with either RPHC or RHHC showed significant reductions in the size of atherosclerotic lesions compared to those with AHHC ($69.5 \pm 1.1\%$ vs. $55.7 \pm 3.4\%$, $P < 0.01$ or $65.6 \pm 1.3\%$ vs. $55.7 \pm 3.4\%$, $P < 0.01$). Reduction of plaque size in the aortic sinus and descending aorta correlated with alterations in cellular immune responses when compared with controls.

Conclusion: We conclude that all recombinant constructs are highly antigenic, among them RPHC may provide new antigenic and structural features which are favorable for significant reduction in atherosclerotic lesion formation. This approach offers a novel strategy for developing anti-atherosclerotic agents.

Disclosure of Interest: None declared.

LATE BREAKING ABSTRACT SESSION

Thrombosis and Anticoagulation

LB001

Screening for occult malignancy in patient with unprovoked venous thromboembolism: an open randomized controlled trial using a comprehensive abdomen/pelvis computed tomography (some trial)

Carrier M¹, Lazo-Langner A², Shivakumar S³, Tagalakis V⁴, Zarychanski R⁵, Solymoss S⁴, Routhier N⁶, Douketis J⁷, Danovitch K¹, Lee AY⁸, Gal GL¹, Wells PS¹, Ramsay T¹, Coyle D¹, Chagnon I⁶, Kassam Z⁹, Taves D⁹, Rodger MA¹, Tao H¹⁰ and On behalf of SOME investigators

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Background: Venous thromboembolism (VTE) may be the earliest sign of cancer. There is presently no standard of care and great diversity in practices regarding occult malignancy screening after unprovoked VTE.

Aims: We sought to assess the efficacy of an occult malignancy screening strategy using a comprehensive abdomen/pelvis computed tomography (cCT) in patients with a first unprovoked VTE.

Methods: Multicenter open-label randomized controlled trial in 9 Canadian sites (NCT00773448). Patients were randomized to undergo limited occult cancer screening (basic blood work, chest X-ray and breast/cervical/prostate cancer screening) only, or limited occult cancer screening in combination with a cCT. The cCT includes: 1) a virtual colonoscopy and gastroscopy; 2) a biphasic enhanced CT; 3) parenchymal pancreatogram; and 4) a uniphasic enhanced CT of distended bladder. Patients were followed for 12 months. The primary outcome measure is previously undiagnosed malignancy missed by the screening strategy and defined as biopsy-proven malignancy confirmed between the time of malignancy screening completion and the end of the follow-up period.

Results: Of the 862 randomized patients, 67% were males and the mean age was 54 years old. Patients presented with symptomatic deep vein thrombosis, pulmonary embolism or both in 55%, 32%, and 13%, respectively. Forty eight percent of patients had a prior smoking history (15% current smoker). Ninety two percent of patients randomized to the cCT arm underwent the procedure. To date, the 1-year incidence of malignancy is 4.3%, with 122 patients continuing follow-up.

Conclusion: The SOME trial is the largest randomized controlled trial assessing the efficacy of extensive occult malignancy screening in patients with unprovoked VTE. The final follow-up of the last included patient is scheduled for April 15th 2015. Final analyses will be submitted to the Congress Office of the ISTH prior to May 19th 2015.

Disclosure of Interest: None declared.

LB002

Bridging anticoagulation in patients who require temporary interruption of warfarin therapy for an elective invasive procedure or surgery (the bridge trial)

Douketis J¹, Spyropoulos A², Kaatz S³, Caprini J⁴, Dunn A⁵, Garcia D⁶, Jacobson A⁷, Jaffer A⁸, Kindzelski A⁹, Schulman S¹⁰, Turpie AG¹¹, Becker R¹², Clark NP¹³, Conti B¹⁴, Ellsworth S¹⁵, Harrison RW¹⁶, Kong D¹⁶, Johnson G¹⁷, Krishnamoorthy A¹⁶, Palmeri S¹⁸, Parker W¹⁶, Saucedo J¹⁹, Schoch P²⁰, Tallman D²¹, Witt D²², Hasselblad V¹⁶, Ortel TL^{2,3} and On behalf of for the BRIDGE Study Investigators

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Background: The management of patients with atrial fibrillation (AF) on warfarin who need treatment interruption for surgery/procedure is a common clinical problem. Bridging with low-molecular-weight heparin (LMWH) has been used to minimize the time that patients are not anticoagulated to mitigate the risk for arterial thromboembolism (ATE). The efficacy of bridging has not been validated, and bridging may increase bleeding.

Aims: We aimed to determine the efficacy and safety of bridging anticoagulation.

Methods: BRIDGE is a randomized, double-blind, placebo-controlled trial comparing bridging vs. no bridging in adults with non-valvular/valvular AF or atrial flutter who required warfarin interruption for elective surgery/procedure. Patients with a mechanical heart valve or creatinine clearance < 30 mL min⁻¹ were excluded. After stopping warfarin 5 days pre-procedure, patients received dalteparin, 100 IU kg⁻¹, or matching placebo, both subcutaneous twice daily, for 3 days pre- and 5–9 days post-procedure. Dalteparin/placebo was resumed 12–24 h after minor and 48–72 h after major surgery/procedure. Warfarin was resumed ≤ 24 h post-procedure. Follow-up was for 30 ± 7 days post-procedure. Primary outcomes were ATE and major bleeding; secondary outcomes were minor bleeding, death, myocardial infarction, and venous thromboembolism.

Results: We enrolled 1884 patients: median age was 72.7 (IQR 65.8–78.2) years; 73.4% were male; median CHADS₂ score = 2.0 (IQR 2.0–3.0); and 336 (17.7%) had prior stroke or transient ischemic attack. Protocol adherence occurred in pre- and post-procedure periods in 1432 (81.0%) and 1669 (94.5%) patients, respectively. We lost 5 (0.3%) patients to follow-up. Trial enrollment ended in Dec. 2014, with results to be presented at the Congress.

Conclusion: BRIDGE is the first randomized trial comparing the efficacy and safety of LMWH bridging vs. no bridging in patients with AF who require warfarin interruption for an elective surgery/procedure.

Disclosure of Interest: J. Douketis: None declared, A. Spyropoulos: None declared, S. Kaatz Consultant for: Boehringer Ingelheim, Bristol Myers Squibb/Pfizer, Janssen/Johnson and Johnson, Daiichi Sankyo,

Speaker Bureau of: Janssen, Boehringer-Ingelheim, Bristol Myers Squibb/Pfizer, CSL Behring, J. Caprini Paid Instructor at: Sanofi Poland, Speaker Bureau of: Janssen R & D, Pfizer, BMS, A. Dunn: None declared, D. Garcia Consultant for: Daiichi Sankyo, BMS, Pfizer, Boehringer Ingelheim, Portola, Genzyme, A. Jacobson Grant/Research Support from: Eisai Co., Ltd, Pfizer, Inc., A. Jaffer Consultant for: Boehringer-Ingelheim, Janssen Pharmaceuticals, Pfizer, BMS, Medtronic, Daiichi Sankyo, Astra Zeneca, Employee of: Rush University Medical Center, A. Kindzelski Employee of: Employee of U.S. Federal Government, S. Schulman: None declared, A. Turpie: None declared, R. Becker Speaker Bureau of: Portola, Daiichi-Sankyo, Boehringer-Ingelheim, Janssen, N. Clark: None declared, B. Conti: None declared, S. Ellsworth: None declared, R. Harrison: None declared, D. Kong Grant/Research Support from: International Business Machines, Cardiovascular Systems, Inc; OrbusNeich Medical, Consultant for: Allmed Healthcare, American College of Cardiology Foundation, Speaker Bureau of: The Medicines Company, G. Johnson: None declared, A. Krishnamoorthy Grant/Research Support from: Novartis, Medtronic, S. Palmeri: None declared, W. Parker: None declared, J. Saucedo: None declared, P. Schoch: None declared, D. Tallman: None declared, D. Witt: None declared, V. Hasselblad: None declared, T. Ortel Grant/Research Support from: Eisai Co., Ltd; Pfizer, Inc.

LB003

Raps (rivaroxaban in antiphospholipid syndrome): a prospective randomised controlled phase II/III clinical trial of rivaroxaban vs. warfarin in patients with thrombotic antiphospholipid syndrome, with or without SLE

Cohen H^{1,2}, Hunt B^{3,4}, Efythymiou M², Arachchilage D², Ruiz M^{5,6}, Clawson S⁷, Muirhead N⁷, Sylvestre Y⁷, Machin S², Bertolaccini ML^{5,6}, Dore C⁷, Mackie I², Isenberg D^{8,9}, Khamashta M^{5,6} and On behalf of the RAPS Trial Collaborators

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Background: Rivaroxaban has been shown to be effective and safe compared with warfarin for the treatment of venous thromboembolism (VTE) in major phase III prospective randomised controlled trials (RCT), but the results may not be directly generalisable to patients with antiphospholipid syndrome (APS).

Aims: The primary aim of RAPS (<http://www.isrctn.com/ISRCTN68222801>) is to demonstrate, in patients with APS and previous VTE, that the intensity of anticoagulation achieved with rivaroxaban is not inferior to that of warfarin. Secondary aims are to compare rates of recurrent thrombosis and bleeding, and the quality of life in patients on rivaroxaban with those on warfarin.

Methods: RAPS is a phase II/III prospective, non-inferiority RCT in which eligible patients with APS, who are on warfarin, target International Normalised Ratio (INR) 2.5 for previous VTE, are randomised either to continue warfarin (standard of care) or to switch to rivaroxaban 20 mg daily. Intensity of anticoagulation is assessed using thrombin generation (TG), with the endogenous thrombin potential (ETP) as the key parameter. The primary outcome is the percentage change in ETP from randomisation to day 42. Markers of *in vivo* coagulation activation, prothrombin fragment 1.2, thrombin-antithrombin complex and D-dimer, will also be measured.

Results: Results will be submitted as soon as available, and presented at the meeting.

Conclusion: If RAPS demonstrates that i) that the anticoagulant effect of rivaroxaban is not inferior to that of warfarin using the TGT; and ii) the absence of any adverse effects that cause concern with regard to the use of rivaroxaban, this would provide sufficient supporting information to make rivaroxaban a standard of care for the treatment of APS patients with previous VTE, requiring a target INR of 2.5.

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Disclosure of Interest: H. Cohen Grant/Research Support from: Bayer, Speaker Bureau of: Bayer, B. Hunt: None declared, M. Efythymiou: None declared, D. Arachchilage: None declared, M. Ruiz: None declared, S. Clawson: None declared, N. Muirhead: None declared, Y. Sylvestre: None declared, S. Machin: None declared, M. Bertolaccini: None declared, C. Dore: None declared, I. Mackie: None declared, D. Isenberg: None declared, M. Khamashta: None declared.

LB004

ANNEXA™-A PART 2: A phase 3 randomized, double-blind, placebo-controlled trial demonstrating sustained reversal of apixaban-induced anticoagulation in older subjects by andexanet ALFA (PRT064445), a universal antidote for factor XA (FXa) inhibitors

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Background: Direct FXa inhibitors appear to have superior or comparable anticoagulant efficacy and safety relative to warfarin. However, a specific antidote for these agents is lacking in case of major bleeding. Andexanet alfa (AnXa) is a modified, recombinant human FXa molecule under clinical development as a specific antidote for FXa inhibitors. We have recently reported data for Part 1 of the Phase 3 registration study in older subjects anticoagulated with apixaban, where an AnXa IV bolus rapidly and significantly reversed anti-FXa activity and restored thrombin generation. Here we report data from Part 2 of this study where AnXa was administered as a bolus *plus* an infusion regimen in a similar study population.

Aims: To demonstrate immediate and sustained reversal of apixaban anticoagulation following administration of AnXa.

Methods: ANNEXA™-A is a Phase 3, double-blind, placebo-controlled study of AnXa in older subjects treated with apixaban. Part 2 investigated a bolus of AnXa followed by a 2-hr continuous infusion. In Part 2, 34 subjects age 50 to 75 were randomized to receive either AnXa or placebo in a 3:1 ratio. All subjects received apixaban 5 mg PO BID for 4 days to achieve steady state plasma levels. AnXa (400 mg IV bolus followed by a 2-hr infusion at 4 mg min⁻¹) or placebo was administered on Day 4, 3 h after the last apixaban dose (~apixaban C_{max}). Safety data were collected through Day 43. The primary efficacy endpoint is the percent change from baseline in anti-FXa activity at its nadir between 10 min prior to and 5 min after the end of the continuous infusion. Additional efficacy endpoints included reduction in plasma free fraction of apixaban and restoration of thrombin generation.

Results: Part 2 of the study has been completed. Efficacy and safety results will be presented and discussed.

Conclusion: This study continues our investigations of AnXa as an antidote for reversing the anticoagulant effects of apixaban and other FXa inhibitors.

Disclosure of Interest: M. Crowther Grant/Research Support from: the Heart and Stroke Foundation of Ontario, Leo Pharma, Consultant for: Janssen, Leo Pharma, Portola, AKP America, Speaker Bureau of: Leo Pharma, Bayer, Celgene, Shire and CSL Behring, A. Gold Employee of: Portola Pharmaceuticals, Inc., G. Lu Employee of: Portola Pharmaceuticals, Inc., J. Leeds Employee of: Portola Pharmaceuticals, Inc., B. Wiens Employee of: Portola Pharmaceuticals, Inc., V. Mathur Consultant for: Portola Pharmaceuticals, Inc., J. Castillo Employee of: Portola Pharmaceuticals, Inc., P. Conley Employee of: Portola Pharmaceuticals, Inc., S. Connolly Consultant for: Portola Pharmaceuticals, Inc., J. Curnutte Shareholder of: 3-V Biosciences, Consultant for: Sea Lane Biotechnologies, Employee of: Portola Pharmaceuticals, Inc.

LB005

Initial results of the re-verse AD trial: idarucizumab reverses the anticoagulant effects of dabigatran in patients in an emergency setting of major bleeding, urgent surgery, or interventions

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Background: Dabigatran, an oral thrombin inhibitor, is widely used for stroke prevention in atrial fibrillation. Idarucizumab, a humanized Fab fragment directed against dabigatran, demonstrated immediate, complete and sustained reversal of the anticoagulant effect of dabigatran, without promoting thrombin generation, in human volunteers.

Aims: To evaluate the safety and efficacy of idarucizumab to rapidly reverse the anticoagulation effects of dabigatran in critical clinical situations.

Methods: RE-VERSE AD is an ongoing multinational, open-label, single cohort study with a target size of 250–300 patients. It is designed to study the reversal of dabigatran's anticoagulant effect by 5 g idarucizumab given as two 2.5 g IV bolus infusions, in patients who experience life-threatening or uncontrolled bleeding (Group A) or who require emergency surgery or procedure (Group B). The primary endpoint is the maximum reversal of the anticoagulant effect of dabigatran, based on central laboratory determination of the dilute thrombin time or ecarin clot time. Drug levels and other measures of dabigatran activity are also performed. Clinical outcomes, including cessation of bleeding, thrombotic events, and other safety measures, are assessed.

Results: Over 50 patients have been enrolled to date; about half in each group. A pre-specified interim analysis has been performed as part of the regulatory approval process. In the first 26 patients, idarucizumab restored and sustained clotting times in the normal range in over 90% of patients. Updated results will be reported.

Conclusion: Idarucizumab is a promising specific reversal agent for dabigatran that rapidly and safely reverses its anticoagulant effects in the most emergency situations.

Disclosure of Interest: C. Pollack Consultant for: Boehringer-Ingelheim, Janssen, BMS/Pfizer, Daiichi-Sankyo, P. Reilly Employee of: Boehringer-Ingelheim, P. Verhamme Grant/Research Support from:

Boehringer-Ingelheim, Consultant for: Boehringer-Ingelheim, R. Bernstein Grant/Research Support from: Boehringer-Ingelheim, Medtronic, Consultant for: Boehringer-Ingelheim, Medtronic, Speaker Bureau of: Boehringer-Ingelheim, Pfizer/BMS, Medtronic, R. Dubiel Employee of: Boehringer-Ingelheim, J. Eikelboom Consultant for: Bayer, Boehringer-Ingelheim, Janssen, BMS/Pfizer, Daiichi-Sankyo, M. Huisman Consultant for: Boehringer-Ingelheim, E. Hylek Consultant for: Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Daiichi Sankyo, Janssen, Medtronic, Pfizer, Roche, C.-W. Kam: None Declared, P. Kamphuisen Consultant for: Boehringer-Ingelheim, Daiichi-Sankyo, LeoPharma, J. Kreuzer Employee of: Boehringer-Ingelheim, J. Levy Consultant for: Jansen, Portola, Roche, The Medicines Company, F. Sellke Consultant for: Boehringer-Ingelheim, T. Steiner Consultant for: Boehringer-Ingelheim, Speaker Bureau of: Boehringer-Ingelheim, B. Wang Employee of: Boehringer-Ingelheim, J. Weitz Consultant for: Boehringer-Ingelheim.

Bleeding disorders, TTP

LB006

Additional data from the titan trial with the anti-VWF nanobody caplacizumab in the treatment of acquired TTP

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Background: Caplacizumab (CAP) is an anti von Willebrand Factor (vWF) Nanobody that is in development for the treatment of acquired thrombotic thrombocytopenic purpura (TTP). CAP binds to the A1 domain of vWF preventing vWF-mediated platelet aggregation characteristic of TTP. The efficacy and safety of CAP in conjunction with standard of care (plasma exchange (PE) and immunosuppressants) was evaluated in the TITAN study in acquired TTP.

Aims: Primary endpoint: time to confirmed platelet response (platelets $\geq 150,000 \mu\text{L}$, confirmed after 48 h).

Methods: Treatment in TITAN consisted of one intravenous bolus injection of CAP or placebo (PLC) prior to first on-study PE, followed by daily subcutaneous administrations of 10 mg CAP or PLC after each PE session and for 30 days following the last PE.

Results: The intent-to-treat population consisted of 36 CAP and 39 PLC subjects. The median time to platelet response for the non-stratified population was significantly faster in the CAP (2.97 days) vs PLC group (4.79 days). The overall hazard ratio was 2.2. The relevance of achieving the primary endpoint with CAP in terms of more rapid curtailment of tissue ischemia is currently being analysed (analysis of organ damage marker normalisation, e.g. LDH, troponin, ...), together with other additional analysis on PE outcome (e.g. number of consecutive days PE and plasma volumes) and 1-month follow-up data.

Conclusion: These additional results will be submitted as soon as available, and will be presented at the meeting.

Disclosure of Interest: F. Peyvandi Grant/Research Support from: Ablynx NV - Investigator fees, M. Scully Grant/Research Support from:

Ablynx NV - Investigator fees, P. Knöbl Grant/Research Support from: Ablynx NV - Investigator fees, J. A. Kremer Hovinga Grant/Research Support from: Ablynx NV - Investigator fees, H. Wu Grant/Research Support from: Ablynx NV - Investigator fees, S. Cataland Consultant for: Ablynx NV, D. Tersago Employee of: Ablynx NV

LB007

Timing of plasma transfusion and maternal mortality and severe maternal morbidity in women with persistent postpartum haemorrhage: the Tempoh-1 study

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Background: In the treatment of major bleeding, correction of coagulopathy by administering plasma is eventually inevitable. For women with persistent postpartum haemorrhage (PPH), however, it is unclear at which point in treatment is indicated plasma transfusion to prevent adverse maternal outcomes.

Aims: To assess whether early plasma transfusion leads to a decrease in maternal mortality and severe maternal morbidity in women with persistent PPH, compared to plasma transfusion later on in treatment.

Methods: This cohort study comprised women from 62 Dutch hospitals that, in 2011 and 2012, received either ≥ 4 units of red blood cells or a multicomponent transfusion within 24 h following delivery due to PPH (≥ 1000 mL). For this analysis, we selected women with persistent PPH, defined as PPH refractory to first-line uterotonic or surgical therapy. Depending on cause of bleeding, baseline was defined as time of first-line therapy or time of delivery. Primary endpoint was a composite of maternal mortality, arterial embolisation, hysterectomy and intensive care unit admission. To account for both time-fixed and time-dependent prognostic factors (i.e. rate of blood loss), we will construct marginal structural models with inverse probability of treatment weighting to quantify the difference in maternal outcomes between an early and a late start of plasma transfusion in women with persistent PPH.

Results: Detailed information has been collected on the course of bleeding and timing of interventions in 1192 women with persistent PPH: median bleeding rate at baseline was 1.1 L h^{-1} (interquartile range 0.6–2.2), median total blood loss was 3.0 L (IQR 2–4.5) and primary endpoints were seen in 33.4% of the study population. With the ongoing statistical modelling, we will answer our research question without the survival bias previously encountered by other authors, whilst dealing with time-dependent confounding according to the latest methodological insights. Results will be submitted as soon as available.

Conclusion: NA.

Disclosure of Interest: None declared.

LB008

rVIII-Singlechain, results of the pivotal phase I/III PK, efficacy and safety clinical trial in adults and adolescents with severe hemophilia A

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Background: rVIII-SingleChain is a novel B-domain truncated recombinant Factor VIII (rFVIII), with a covalent bond between the FVIII heavy and light chains. rVIII-SingleChain has a higher binding affinity to von Willebrand Factor (vWF), a lower clearance, longer half-life, higher mean residence time and larger area under the curve compared to rFVIII (octocog alfa).

Aims: This Phase I/ III study investigated the PK, safety and efficacy of rVIII-SingleChain in patients with severe Hemophilia A in the treatment and prevention of bleeding episodes.

Methods: The study was approved by the relevant Ethics committee and national authorities and conducted according to ICH-GCP and the Declaration of Helsinki. 175 patients with severe Hemophilia A were enrolled, 173 entered the treatment period. Patients underwent on-demand or prophylaxis treatment with dosing guided by the WFH recommendations.

Results: Final results will be presented in the late breaking clinical trial submission in May. Preliminary: The study accumulated more than 14293 exposure days in 146 patients on prophylaxis and 27 patients that have been treated on demand. 120 patients were treated for more than 50 and 54 for more than 100 exposure days. Of the 830 bleeds that have been treated and evaluated by the investigator, the efficacy of rVIII-SingleChain was rated excellent or good in 94%. Of the patients receiving prophylaxis therapy, 6% were dosed every other day, 54% were dosed $3 \times$ week, 32% were dosed $2 \times$ week and 8% followed other dosing regimens. The median ABR in the prophylaxis group was 1.66. No inhibitor development was observed in the study.

Conclusion: rVIII-SingleChain is a novel rFVIII without glycopegylation or fusion to antibody fragments, showing superior pharmacokinetics compared to octocog alfa, excellent efficacy and a positive safety profile in a large clinical study.

Disclosure of Interest: J. Mahlangu: None declared, K. Kuliczowski: None declared, O. Stasyshyn: None declared, A. Skotricki: None declared, M. Kasinova: None declared, D. Bensen Kennedy Employee of: CSL Behring, N. France Employee of: CSL Behring, K. St.Ledger Employee of: CSL Behring, I. Pabinger: None declared.

LB009

Safety, efficacy and pharmacokinetics of recombinant factor IX FC fusion protein in children with haemophilia B (KIDS B-LONG)

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Background: Early prophylactic replacement of factor IX (FIX) improves long-term clinical outcomes in children with haemophilia B. Due to the relatively short half-lives of conventional FIX products, intravenous injections up to 3 times/week are often necessary; children may require more frequent dosing, as age has been shown to contribute to variability in factor clearance and half-life. The frequency of administration can be a barrier to prophylaxis. In the B-LONG study recombinant FIX Fc fusion protein (rFIXFc) had a prolonged half-life (82.1 hr) compared with rFIX in adults and adolescents with haemophilia B, allowing for reduced infusion frequency.

Aims: Kids B-LONG was an international, multicenter, open-label prospective phase 3 study that evaluated the safety, efficacy, and pharmacokinetics (PK) of rFIXFc in previously treated patients aged < 12 years with severe haemophilia B (≤ 2 IU dL⁻¹ endogenous FIX).

Methods: Inclusion criteria were ≤ 2 IU dL⁻¹ endogenous FIX, ≥ 50 prior exposure days (EDs) to FIX, and absence of a history of inhibitors to FIX. Planned enrolment was approximately 26 subjects (~13 aged < 6 years and ~13 aged 6 to < 12 years) dosed with rFIXFc, to achieve a minimum of 10 subjects in each age cohort with at least 50 EDs and adequate data for sequential PK evaluations with pre-study FIX (50 IU kg⁻¹), followed by rFIXFc (50 IU kg⁻¹). Prophylactic treatment was started at a dose of 50–60 IU kg⁻¹ rFIXFc weekly, with dose and interval adjustments based upon PK data and bleeding frequency. The primary endpoint was development of inhibitors (neutralizing antibodies). Key secondary endpoints included PK, annualized bleeding rate (ABR), and number of infusions required to control a bleed.

Results: Safety, efficacy and PK results will be submitted when available as a late-breaking clinical trial.

Conclusion: The final analysis of the Kids B-LONG study will provide further PK information and evaluate the safety and efficacy of rFIXFc in children.

Disclosure of Interest: K. Fischer Grant/Research Support from: Bayer, Baxter, Wyeth/Pfizer, Novo Nordisk, Consultant for: Bayer, Baxter, Biogen Idec, Novo Nordisk, Pfizer, Speaker Bureau of: Bayer, Baxter, CSL Behring, Pfizer, Novo Nordisk, R. Kulkarni Grant/Research Support from: Biogen Idec, Novo Nordisk, Octapharma, Consultant for: Biogen Idec, Novo Nordisk, Baxter, Bayer, Pfizer, Cangene, B. Nolan Grant/Research Support from: Biogen Idec, J. Mahlangu Grant/Research Support from: Biogen Idec, Bayer, CSL Behring, Novo Nordisk, Consultant for: Amgen, Bayer, Genentech, Novo Nordisk, S. Rangarajan Grant/Research Support from: Pfizer, Grifols, Baxter, Consultant for: Grifols, Biogen Idec, G. Gambino Shareholder of: Biogen Idec, Employee of: Biogen Idec, L. Diao Shareholder of: Biogen Idec, Employee of: Biogen Idec, L. Cristiano Shareholder of: Biogen Idec, Employee of: Biogen Idec, G. Pierce Shareholder of: Biogen Idec, G. Allen Shareholder of: Biogen Idec, Employee of: Biogen Idec.

LB010

Update on a phase 1/2 open-label trial of BAX335, an adeno-associated virus 8 (AAV8) vector-based gene therapy program for hemophilia B

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Background: Hemophilia B is a bleeding disorder due to a deficiency of coagulation factor IX (FIX). About 40% of patients have < 1% of normal plasma FIX levels, with recurrent bleeding episodes. Gene therapy, by enabling persistent endogenous production of FIX, may provide long-term benefit with a single administration. Non-integrating adeno-associated viral (AAV) vectors have shown to induce stable transgene expression with an excellent safety profile. Baxter Healthcare has developed a gene therapy product BAX335 (AAV8.sc-TTR-FIXR338Lopt): a codon optimized hyperactive FIX transgene (FIXR338Lopt), driven by the liver-specific transthyretin (TTR) promoter in an AAV8 capsid.

Aims: Evaluate plasma FIX activity and its relationship to vector dose; determine dose to achieve stable plasma FIX activity between 10% and 40% of normal; evaluate systemic immune responses to FIXR338Lopt and the AAV8 capsid.

Methods: Up to 16 male adults with severe hemophilia B are to be given a single intravenous dose of BAX335 in up to 4 sequentially ascending dose cohorts. Pharmacodynamic (eg. plasma FIX activity) and safety data (eg. immune response, adverse events [AEs]) are to be collected.

Results: We report data available from the first 6 subjects dosed with BAX335: 2 at 2×10^{11} vg kg⁻¹ (Cohort 1), 3 at 1×10^{12} vg kg⁻¹ (Cohort 2), and 1 at 3×10^{12} vg kg⁻¹ (Cohort 3), with follow-up ranging from 7 weeks to 2 years. None of the subjects developed FIX inhibitors. A dose-dependent immune response with variable neutralizing antibody titers to the AAV8 capsid was observed 2 weeks post gene transfer in all subjects. No product-related AEs were reported. Therapeutic FIX levels of 3% were achieved in Cohort 1, sustained levels of 0.5 to 20% were observed 6 months post dosing in Cohort 2, and sustained levels above 25% were observed in Cohort 3.

Conclusion: BAX335 appears to be well-tolerated in the 6 subjects dosed to date and hemostatically effective plasma FIX levels were achieved in two dosing cohorts.

Disclosure of Interest: None declared.

NURSE AND ALLIED HEALTH SESSION

Bleeding

NUR001

When 2N VWD should be considered a differential diagnosis

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Background: Type 2N von Willebrands disease is a rare bleeding disorder, inherited recessively. It is often mistaken for mild haemophilia A on basic laboratory investigations. However given the inheritance pattern is vastly different, it is a condition that should be considered when a new case of mild haemophilia A presents.

Aims: This collection of case studies aims to highlight how type 2N vWD can be overlooked with patients and their families diagnosed with mild haemophilia A.

Methods: A review of pathology and genetic results together with patient and family histories was undertaken on those with a diagnosis of 2N vWD.

Results: The Haemophilia Centre of WA identified 4 families who had had a diagnosis change and another where investigations were continuing. In some cases failure to respond to treatment as predicted sparked a reassessment of the bleeding disorder. In others the laboratory results were not consistent with a definitive diagnosis of haemophilia and lead to binding assays being performed. In one case it was the family bleeding history and inheritance pattern that was not in keeping with a diagnosis of haemophilia, even though on first examination the laboratory results were indicative of haemophilia A and was diagnosed as such. In some families the change in diagnosis was made with 12 months – however in the majority of these families the re-diagnosis was years after the initial diagnosis, for some more than 35 years. In most of these families this change in diagnosis has affected multiple generations of family members, leading to a re-examination of possible affected family members and perhaps those that are unlikely to now be affected.

Conclusion: Type 2N vWD should be considered as an alternative diagnosis and excluded when a new case of mild haemophilia A presents. It should also be considered when the family inheritance pattern does not correlate with a diagnosis of haemophilia or when treatment outcomes do not correlate with the predicted outcome of haemophilia management.

Disclosure of Interest: None declared.

NUR002

The DDAVP challenge – an audit to assess practice in a UK haemophilia centre

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Background: Administering and managing DDAVP (desmopressin) therapy is an important duty for haemostasis nurses. The UKHCDO

has issued guidelines on DDAVP usage in patients with von Willebrand disease (VWD) and haemophilia A (HA). An audit was carried out on adult patients at St George's Haemophilia Centre (SGHC) who had received DDAVP from 2011–2014. Standard DDAVP dosing at SGHC is with a capped dose of 15 µg (1 ml) given subcutaneously.

Aims: 1. To assess efficacy of a capped dose (15 µg g) of DDAVP in patients with VWD and HA in terms of laboratory and clinical parameters. 2. To obtain views on patient experience with DDAVP.

Methods: A retrospective analysis was performed on 25 patients who received a capped dose of DDAVP for VWD or HA. Diagnosis, dose given, pre and post administration levels, clinical efficacy and side effects of DDAVP were analyzed. A postal questionnaire about knowledge level, benefits and drawbacks of DDAVP was sent to 89 patients who had received DDAVP for any indication; the response rate was 34%.

Results: 18 of 25 patients (72%) achieved FVIII:C and VW activity levels > 50 iu dL⁻¹ after DDAVP. Regression analysis showed no correlation between weight and responsiveness; a T – Test found a non-significant difference ($P = 0.32$) in weights between responders and non-responders. The questionnaire indicated that patients were more familiar with the benefits of DDAVP than the drawbacks; only 57% of respondents knew about fluid restriction. The most commonly reported side effects were flushing and/or headaches. 90% of respondents were satisfied with the care and advice they received in relation to DDAVP.

Conclusion: At SGHC, capped dosing of DDAVP is a clinically effective treatment for the majority of patients with VWD and HA. Patient experience with DDAVP is positive but further education is needed about fluid restriction post administration.

Disclosure of Interest: None declared.

NUR003

Closing the GAP: a multidisciplinary approach developing local services for rural and remote patients with bleeding disorders

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Background: Patients with bleeding disorders in rural and remote areas face well-known challenges to timely and appropriate emergency management. We identified common scenarios in these areas resulting in avoidable transfers to our tertiary centre and developed an innovative program to close this gap in service.

Aims: Our aim was to create a local platform of care to support patients living in rural and remote locations. It included providing professional health care education and support, routine supply of clotting factor concentrate, community awareness to facilitate local treatment by enabling local care partnerships through enhanced lines of interagency communication.

Methods: Four rural areas with a high demand for our services or a large population of patients were identified as pilot sites. In consultation with key local stakeholders, we formed a multidisciplinary outreach team to develop and trial the program consisting of education and community information sessions, onsite in-services for medical, nursing and allied health care professionals with ongoing patient communication and program evaluation.

Results: One pilot site has reached the final stage. The local General Practitioners facilitate acute care coordination. Education of the hospital staff has been successfully completed with new policies and procedures implemented. Since implementation of the program at this site there have been five recorded bleeds requiring intervention, three of which were successfully treated locally. Two required transfer for specialist intervention however received factor prior to transfer. The three remaining locations are well on track and initial activities have

commenced. Local supply of coagulation factor has commenced in all sites.

Conclusion: Rural and remote treatment can be successful. Overall the program not only benefits the patients and their families, but also provides local health care professionals with the required resources, leading to reduced hospital admissions and its associated cost of treatment.

Disclosure of Interest: J. Slade Grant/Research Support from: Novo Nordisk: Changing Possibilities in Haemophilia.

NUR004

Non-adherence to prophylaxis in haemophilia: extent and underlying reason

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Background: Given the lifelong prophylaxis in severe haemophilia, insight in (non) adherence behaviour is important to understand patients' difficulties with following treatment recommendations and optimize support.

Aims: To provide an overview of four studies leading to insight in the extent and the reason for non-adherence to prophylaxis in haemophilia.

Methods: First, consensus on the definition of adherence was achieved through a Delphi procedure. 23 experts (patients and health care providers) agreed (84–95%) on the definition after three rounds. This definition was used to quantify adherence through a short interview during the nursing consultation ($n = 241$). Insight in the underlying reason of non-adherence was obtained through a systematic literature review followed by a qualitative study (grounded theory). This study included Individual in-depth interviews in 21 adults aimed at understanding experiences, perceptions and beliefs of adherence.

Results: The developed definition of adherence involved three aspects: missed infusions (%), changes in dosage/dose changes (%) and infusions not administered at prescribed time point (%). This definition was used to quantify the adherence levels: 66% of the parents administering their child vs. 43% of the patients were adherent. Furthermore, 29% of the parents and 37% of patients were defined as sub-optimally adherent and 5% of the parents and 20% of the patients were non-adherent. The review about barriers and motivators of adherence defined significant factors influencing adherence. The qualitative study identified patterns in the previously identified factors, which led to a consistent model. Adherence was determined by the positions of prophylaxis in life, which was influenced by the level of acceptance of haemophilia and self-management skills.

Conclusion: The present results serve as a baseline for an intervention to improve adherence, focussed on self-management and acceptance issues.

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NUR005

Expanding the knowledge base: opportunities and challenges for allied professionals in bleeding disorder research in Canada

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Background: Practice-based evidence is an important component of allied health professionals' knowledge base and is essential for providing best care to patients with bleeding disorders. According to the Society for Social Work and Research, from 1994–2014, social work produced the fewest researchers and doctoral scholars among peer disciplines. Thus we need additional ways to encourage knowledge development.

Aims: Our aim is to understand the current capacity of social workers at Canadian hemophilia treatment centres to initiate, participate in and publish research.

Methods: There are 24 hemophilia treatment sites across Canada, which employ 20 social workers on a part-time basis (0.1 to 0.6 FTEs) to provide psychosocial support to individuals with bleeding disorders. We polled these participants electronically. Open-ended questions about the extent of, prospects for, and barriers to social work-led research elicited responses which were analyzed qualitatively.

Results: We will describe the extent to which social workers currently direct or participate in bleeding disorder-related research, their willingness to play a greater role and perceived benefits of so doing. Organizational barriers to this include the scarcity of psychosocial grants in hemophilia, and lack of protected time/coverage to engage in research. Individual-level barriers include comfort with subjects like research design or data analysis.

Conclusion: Canadian hemophilia treatment centres and professional organizations should address these identified barriers in order to improve knowledge development and transfer related to bleeding disorders. An absence of social work and allied health perspectives eliminates important forms of practice-based evidence, to the detriment of patient care and frustration of professionals' career aims. Possible actions – for instance, having social work organizations lead in initiating projects and opportunities for skill development – are suggested.

Disclosure of Interest: None declared.

Thrombosis and Anticoagulation

NUR006

Developing a nurse led ultrasound scanning service to confirm or exclude the diagnosis of lower limb deep vein thrombosis - improving patient care and reducing the risk of unnecessary anticoagulants in a United Kingdom primary care setting

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Background: SEQOL provide a nurse-led community deep vein thrombosis (DVT) service for patients within Wiltshire and surrounding counties. An average of 1400 patients are assessed annually, only 14–17% of these are confirmed to have the condition. Between June 2012 and June 2013 20.4% of patients received an ultrasound scan (USS) within 24 h of referral. Four scans were available daily from the secondary care providers with no capacity for peaks and troughs in attendance numbers; the DVT service had an average daily requirement of 4.4 resulting in long waits and the excessive use of LMWH while awaiting definitive diagnosis.

Aims: To develop nurse practitioners as qualified sonographers who can provide point of care duplex USS to confirm or exclude the diagnosis of DVT.

Methods: Initial project work involved engagement with the secondary care scanning department who provided mentorship. The University of the West of England were approached concurrently and a 9 month post graduate study program was devised. One practitioner was trained initially to ensure a portfolio of > 250 supervised USS was achieved.

Results: Since 28/10/14 324 patients have been scanned, 84% within 4 h of referral and 96% within 24 h. All patients receive an USS regardless of clinical probability; blood tests are reserved for patients following a positive diagnosis only. A locum vascular scientist was employed to audit quality and ensure a high clinical standard is maintained. Two further nurse practitioners are currently training, the service will have a scanning capacity of 16 h a day 7 days a week on completion of this training. An annual saving of £19,000 will be achieved due to a reduction in the use of LMWH, further savings in the region of £70,000 are estimated due to reduced staffing needs.

Conclusion: This improved service offers a streamlined patient journey resulting in financial savings while improving safety by reducing the risk of adverse events related to unnecessary anticoagulation.

Disclosure of Interest: None declared.

NUR007

Overweight is a risk factor for venous thromboembolism after total knee arthroplasty despite rivaroxaban thromboprophylaxis

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Background: The roles of rivaroxaban for patients undergoing total knee arthroplasty (TKA) have been established by rigorous clinical trials. Pharmacokinetic data of rivaroxaban for obese persons are conflicting. Since obesity is an independent risk factor for venous thromboembolism (VTE), we hereby evaluated whether body weight was associated with VTE in post-TKA patients despite rivaroxaban prophylaxis.

Aims: To assess whether the body mass index (BMI) in patients complicated by VTE after TKA was different from those uneventfully recovered from the surgery.

Methods: This case-control study was conducted based on a database of an academic thrombosis service from Jan 2013 to Dec 2014. We first identified patients on rivaroxaban prophylaxis but developed VTE within 14 days after TKA. These index patients were matched to randomly selected controls at a 1:3 ratio, who underwent the same type of surgery and received the same prophylaxis. Patients being on other thromboprophylaxis such as mechanical compression, antiplatelet agents or heparins were excluded from both index and control groups. Two investigators independently extracted the patients' medical and surgical data and discrepancy was resolved by consensus. A student's *t*-test was employed to compare the BMI between the case and the control groups. A *p* value of < 0.05 was considered significant.

Results: Within the 2 year study period, 9 index patients were identified. The BMI of these patients were 39.2 (±7.75), which was significantly different from the BMI of the 27 matched controls (33.3 ± 6.22, *P* = 0.03). There was no fatality from VTE.

Conclusion: This study suggests overweight is a risk factor for VTE in the immediate post-operative period after TKA despite rivaroxaban prophylaxis. Our results are opposite to the subgroup analysis of RECORD trials; thus, warrant further exploration. In particular, the time to initiate rivaroxaban was longer in our patient population than those in the RECORD trials.

Disclosure of Interest: None declared.

NUR008

Managing anticoagulation therapy risk through the multidisciplinary team (MDT) ward round at King's college hospital

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Background: Anticoagulant therapy is frequently reported as causing preventable patient harm. In 2008, the anticoagulation MDT established a thrice weekly ward round to manage this risk. A subsequent review following its inception demonstrated significant improvements on documentation of patients' anticoagulation plans and addressing queries regarding patients with complex anticoagulation needs.

Aims: To describe the activity of the MDT ward round in more recent years (07/2012-12/2014), and assess its impact on patient care.

Methods: All patients referred and reviewed to the ward round were recorded on a central database. In addition to demographic information, reasons for referral, any interventions made by the MDT, along with specific advice given was documented. This information was retrospectively reviewed.

Results: Out of 1997 referrals received, 1508 (76%) patients were reviewed by the MDT. The number of referrals and subsequent reviews, has increased year on year, since 2008. The majority of referrals originate from general medicine, care of older adults and the cardiothoracic surgery specialities. A total of 3574 interventions were made for patients reviewed (intervention mean score per patient 3.66); these ranged from counselling patients regarding anticoagulant therapy (882), providing tailored written information (882), the provision of anticoagulation dosing advice (485), in addition to advice for patient's prescribed triple therapy (141) and in the context of cancer (31). In recent months, interventions around the commencement of novel oral anticoagulants have increased; 1% (Jul 2013) to a mean of 17% (Dec 2014).

Conclusion: The MDT ward round is an effective way to educate and answer questions for patients newly commenced on oral anticoagulant therapy and ultimately manage anticoagulation risk. The MDT is a great resource for non-specialists working in the hospital and seamlessly manages risk associated across the tertiary/secondary/primary care interface.

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NUR009

Safe discharge planning for patients on anticoagulants: launch of a new quality improvement tool for nurses

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Background: Anticoagulants (ACs) are commonly used high-alert medications in the prevention and treatment of venous thromboembolism (VTE) or for stroke prevention in patients with atrial fibrillation. Yet, they are not without risk: for example, warfarin is among the medications with the most food and drug interactions, and it is also among the leading medications implicated in ER visits related to adverse drug events in older adults. More recently, new oral anticoag-

ulants (NOACs) have been approved, and their safety implications are no less important given the lack of reversal agents available. It is well-known that patient education is associated with a reduction in adverse events and improved adherence with AC treatment. Thus, nurses have an essential role to play, particularly at the time of hospital discharge.

Aims: To ensure safe management and follow-up for patients discharged on ACs by standardizing the process and coordination of planning and teaching.

Methods: Upon clinical observation of discharge challenges and nursing staff requests for greater support with discharge planning, a multi-disciplinary team (pharmacist, nurses from thrombosis, medicine, surgery and discharge-planning) was convened and an algorithm was created. Teaching sessions were provided, using a multiple-choice pre- and post- quiz on ACs and discharge planning as well as the new algorithm.

Results: 98 nurses from medical-surgical units participated. The overall mean score pre-test was 48%. At baseline, nurses were most knowledgeable regarding which ACs require follow-up at the Anticoagulation Clinic or in the community; staff were least comfortable regarding which teaching materials were available for patients and families, and when Anticoagulation Clinic teaching session for new patients is given. Post-teaching mean scores were 82%.

Conclusion: Use of the algorithm led to increased knowledge regarding safe discharges for patients on ACs.

Disclosure of Interest: None declared.

NUR010

Direct-acting oral anticoagulants in the real world: insights into appropriate prescribing and medication use

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Background: Direct-acting oral anticoagulants (DOACs) are convenient because of fixed dosing without monitoring. There are instructions on avoidance of moisture, no crushing of capsules, and administration with food for some DOACs. Whether patients adhere to this and are prescribed appropriate doses is unknown.

Aims: To assess DOAC dosing and medication use.

Methods: Patients > 18 years old, receiving a DOAC for any diagnosis, were prospectively included. Nurses at our perioperative anticoagulation clinic helped patients complete a 15-item questionnaire on creatinine clearance, DOAC type, dose, prescriber, and use (exposure to moisture, administration with food, and pill crushing). We categorized appropriateness of dose based on primary diagnosis, age, and creatinine clearance; and appropriateness of use based on administration instructions in the product monograph. Nurses' concerns about dose or use were communicated to patients and physicians.

Results: We recruited 93 consecutive patients—49 on dabigatran, 18 on apixaban, and 26 on rivaroxaban. Mean age was 71 (±33) years, 29% were female, 97% had atrial fibrillation, and 3% had venous thrombosis. 64% of all patients had appropriate DOAC dosing and use; 19% used the DOAC appropriately but at an inappropriate (mostly too low) dose; 14% had an appropriate dose but used it inappropriately. Ten patients removed dabigatran from its packaging well before administration, exposing it to moisture; 2 did not take treatment doses of rivaroxaban with food; and 1 crushed the dabigatran capsule. Multinomial logistic regression did not show that age, gender, creatinine clearance and prescriber type influenced appropriateness of dose or administration.

Conclusion: This pilot study demonstrates large variability in how DOACs are dosed, and how patients take them. Improved medication literacy around DOACs is needed. Our study highlights opportunities

that nurses have to improve patients' medication literacy – even during a brief clinic visit – and alert physicians to dosing errors.

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NUR011

Novel oral anticoagulant checklists for front line clinicians

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Background: Based on their clinical efficacy, safety and ability to overcome many challenges associated with warfarin, apixaban, dabigatran, and rivaroxaban have become accepted options for atrial fibrillation and venous thromboembolism treatment and prophylaxis. With rapidly evolving literature surrounding these agents, it is paramount that clinicians have access to up to date information to make informed choices for the population they serve.

Aims: Succinct checklists titled “Is [Generic name (Trade Name)] an Option for Your Patient?” were created for apixaban, dabigatran, and rivaroxaban. The goal of the checklists was to enable clinicians to make informed decisions regarding the appropriateness of therapy, dose and management of commonly encountered scenarios.

Methods: Action: A group of 10 pharmacists with a clinical interest/focus in thrombosis developed the checklists using primary literature, product monographs, and clinical practice guidelines. The checklists summarize indications, contraindications, advantages, limitations, dosing recommendations, patient information, monitoring, switching between anticoagulant agents and peri-procedural management. The checklists were initially disseminated by various means (conference presentations, peer to peer) and are now primarily accessed online through the RxFiles website (rxfiles.ca).

Results: Evaluation: The developers review/revise the checklists at minimum every 6 months or more frequently if warranted, based on new and evolving literature. Each checklist is downloaded from the RxFiles website on average 201 times per month (range: 139–255). Informal feedback from end users (pharmacists and physicians) has been positive.

Conclusion: Front line clinicians require quick access to accurate information to make safe and effective decisions regarding new and evolving anticoagulant options for their patients. These checklists provide an up to date summary of relevant information to prescribe and manage dabigatran, rivaroxaban and apixaban.

Disclosure of Interest: J. Lowerison: None declared, J. Bolt: None declared, T. Bungard Consultant for: Speaking Engagements; no personal financial gain, D. Lamb: None declared.

NUR012

A Cross-sectional registry on the incidence of gastrointestinal associated symptoms with pradaxa use (Gasp Registry)Zondag MM¹, Sardo L² and Schulman S¹¹Medicine, McMaster University; ²Thrombosis Service, St. Joseph's Healthcare, Hamilton, Canada

Background: The incidence of gastrointestinal symptoms (GIS) on dabigatran was 15.5% in a phase III trial and 2.1% of subjects stopped the drug due to this.

Aims: To study the incidence of GIS in patients on dabigatran. To determine techniques they use to treat GIS and their effectiveness.

Methods: In a cross-sectional study for adult patients on/ever on dabigatran at Hamilton Health Sciences we administered a questionnaire in person or via telephone. GIS included heartburn, indigestion, regurgitation, excessive burping, nausea, vomiting, diarrhea, lower abdominal pain or bloating, and gas. We captured symptom type and severity using a Likert grading scale. The validated Short Form Leeds Dyspepsia questionnaire (SF-LDQ) was used for accurate description of GIS symptoms.

Results: We recruited 119 subjects with an average age of 74 ± 11 years. All subjects had atrial fibrillation, 38% were female, 50% took 110 mg bid, 49% took 150 mg bid and one took 75 mg bid. 83% were currently taking dabigatran; 2 had stopped due to GIS. 65% of patients reported GIS at some point while taking dabigatran. The most troublesome symptom reported most frequently on the SF-LDQ was indigestion (18%) followed by heartburn (15%), nausea (10%) and regurgitation (7%). A total score of the frequency and severity responses for each symptom (maximum possible score 32) from the SF-LDQ was evaluated against confounding factors; no association between prevalence of GIS and weight ($P = 0.5$), ulcer history ($P = 0.18$), aspirin use ($P = 0.2$), dabigatran without meals ($P = 0.4$). History of reflux was borderline significant ($P = 0.047$) and irritable bowel syndrome (IBS) was significantly associated with increased prevalence of GIS ($P = 0.024$). 40% used antacids to manage GIS and reported that most of symptoms resolved, 2% used food or milk with similar relief.

Conclusion: These findings suggest that a history of IBS predicts occurrence of GIS in the setting of dabigatran, and that antacid use is the most common remedy for GIS and provides good relief of symptoms.

Disclosure of Interest: M. Zondag: None declared, L. Sardo: None declared, S. Schulman Grant/Research Support from: Boehringer Ingelheim, Consultant for: Boehringer Ingelheim.

NUR013

Assessing oral anticoagulant use in long-term care residents: evidence for ongoing suboptimal use in 2014.Rojas-Fernandez C^{1,2}, Zarrin A¹, Warkentin M¹, Bonneau J¹, Hartwick J³ and Brown S²¹School of Pharmacy, University of Waterloo; ²RIA, UW-Schlegel Research Institute for Ageing; ³Schlegel Villages Support Office, Schlegel Villages, Kitchener, Canada

Background: The safe and appropriate use of oral anticoagulants (OACs) remains a challenge in older people. Use of OACs is common for residents of long term care (LTC) facilities, yet contemporary studies demonstrate that OACs are underused, and in the case of warfarin, residents spend ~50% of the time outside of the therapeutic range, placing them at risk for adverse events.

Aims: To characterize current patterns of OAC use in LTC residents and describe the quality of warfarin use.

Methods: Retrospective chart review (Aug 2013 to Sept 2014) of residents of 13 LTC facilities (census 3309) who had documented indica-

tions for OACs. The time in therapeutic INR range (TTR, Rosendaal's method) was the primary outcome. This project was approved by the University of Waterloo Office For Research Ethics; individual patient consent was not required.

Results: 564 residents (70% female) with an average age of 84 years were identified. Participants had an average of 7.5 comorbidities and 9 medications. Indications for OAC use were atrial fibrillation (63%), venous or pulmonary embolism (16%), cardiac valves (0.5%); 25% did not have documented indications. OACs were not prescribed for 147 (26%) of residents with indications for OACs. Warfarin was prescribed for 275 (66%) residents; the remainder received novel OACs. The TTR ranged from 56% to 75% (mean 63%) across 13 facilities; the average TTR for the six facilities with the lowest TTRs was 59%. The frequency of INR determinations ranged from every 7 to 19 days, (mean every 13 days) with no apparent relationship between frequency of testing and TTR.

Conclusion: The TTR for was higher (63.8%) than the literature average (50%), but is suboptimal given the expected benefits of TTRs > 75% vs TTRs circa 60%. Documentation of indications for OACs needs improvement, and it is possible that OACs are underused, though reasons for this are presently unclear. Analyses are ongoing to describe adverse events, co-prescribing of interacting medications and other secondary outcomes.

Disclosure of Interest: C. Rojas-Fernandez Grant/Research Support from: Research Grant From BMS, A. Zarrin: None declared, M. Warkentin: None declared, J. Bonneau: None declared, J. Hartwick: None declared, S. Brown: None declared.

NUR014

"Post-thrombotic panic": panic, positive outcomes and the paradoxical impact of venous thromboembolism (VTE)Hunter R¹, Bennett PD¹, Lewis S², Rance J¹ and Noble S²¹Psychology, Swansea University, Swansea; ²Haematology, Aneurin Bevan University Health Board, Wales, UK

Background: The long-term consequences of experiencing venous thromboembolism extend beyond physical phenomena such as post thrombotic syndrome and pulmonary hypertension. Previous research suggests survivors of large volume symptomatic pulmonary emboli later experience elevated levels of distress and anxiety that impact their quality of life, with some demonstrating characteristics akin to post-traumatic stress disorder (PTSD).

Aims: To understand the psychosocial impact of VTE through participants own words.

Methods: Twelve patients with newly diagnosed VTE were recruited through a hematology clinic. Semi structured qualitative interviews were audio recorded and transcribed verbatim. Transcripts were analyzed using interpretative phenomenological analysis (IPA) to identify emergent themes identified.

Results: The content of themes varied according to gender, age at time of VTE and participant's experience of diagnosis and treatment. Emergent themes suggest a paradoxical duality and include: 1. Post-traumatic stress symptoms: Participants described significant symptoms of anxiety and panic, intrusive thoughts and images, and fear of VTE recurrence. 2. Positive outcomes: The experiences were life changing for participants, leading to re-evaluation of life and subsequent changes in self.

Conclusion: Our initial data demonstrates that the post-thrombotic period can be a time of significant psychosocial distress including post-traumatic stress symptomology. It also highlights a duality of trauma and growth after VTE, which suggests a potential role for health psychology in identifying and supporting individuals at risk of post-traumatic stress. Opportunities for impacting patient outcomes via

targeted interventions to enhance psychological wellbeing and recovery are discussed.

Disclosure of Interest: None declared.

Platelet Disorders

OR001

JAK2 V617F mutation and cardiovascular risk factors discriminate young patients with high risk of thrombosis in essential thrombocythemia

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Background: The median age at diagnosis of essential thrombocythemia (ET) is in the sixth decade of life, and patients younger than 40 years account for < 20% of the patients. Information on the outcome of young patients is limited, and whether the calreticulin (CALR) mutations affect the outcome of these patients remains unknown.

Aims: The objective of the study was to assess the clinical outcome of young patients diagnosed with ET and to explore the risk factors for major thrombosis.

Methods: A total of 188 young patients (age ≤ 40 years) with an initial diagnosis of ET were enrolled. Mutations in JAK2 V617F, MPL exon 10 and CALR exon 9 were investigated.

Results: The median follow-up was 61 months (range 0–217). The median age was 35 years (range 18–40). Mutational frequencies were 44.7% for JAK2 ($n = 84$), 27.1% for CALR ($n = 51$) and 1.6% for MPL ($n = 3$). Twenty-six (13.8%) experienced major thrombotic events. Cox proportional hazards regression revealed JAK2 V617F ($HR = 3.241$; $P = 0.008$) and cardiovascular risk factors (CVF) ($HR = 3.646$; $P = 0.001$) to be risk factors for thrombosis. Neither CALR mutation nor previous thrombosis was a risk factor for thrombosis. Kaplan–Meier analysis revealed a worse thrombosis-free survival in JAK2-mutated patients than in patients with wild-type JAK2 ($P = 0.006$), and a worse thrombosis-free survival in patients with CVF than those without ($P = 0.001$). The thrombosis-free survival was much worse in patients with both JAK2 V617F and CVF than in those only with JAK2 V617F or CVF or with neither ($P < 0.001$). Transformation to myelofibrosis was observed in 4 patients (2.1%), and none of the evaluated factors could predict fibrotic transformation ($P > 0.05$).

Conclusion: Different from the general ET population, previous thrombosis is not a risk factor for thrombosis in young patients. JAK2 V617F and CVF can discriminate a high-risk group of young patients, while the CALR mutation is not a risk factor for thrombosis or for fibrotic transformation.

Disclosure of Interest: None declared.

OR002

First report of a new homozygous FLI1 mutation unraveled by increased MYH10 expression in an inherited platelet disorder

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Background: Inherited platelet disorders (IPD) comprise multiple diseases affecting platelet production, morphology and/or function. Standard laboratory investigations can only diagnose < 20% of IPD and new approaches need to be implemented to help with diagnosis. We report here the case of 2 Caucasian sisters who both presented with thrombocytopenia (platelets 40–90 × 10⁹/L) and bleeding history. Platelet aggregation to ADP and adrenalin was mildly reduced but normal to arachidonic acid and ristocetin. Collagen-induced platelet aggregation was absent. Collagen receptor genes were sequenced but no abnormality was detected.

Aims: To use protein analysis and DNA sequencing to investigate this uncharacterized platelet disorder.

Methods: Informed consent was obtained and blood collected for platelet function testing, platelet lysate for western-blotting (WB), electron microscopy (EM) and DNA extraction. Massively parallel sequencing (MPS) was performed using MiSeq Illumina instrument and a 19 gene panel.

Results: WB was performed to quantitate granule and cytoskeleton proteins. MYH10 expression was increased in platelets from both sisters in contrast to normal platelets where MYH10 is suppressed. MYH10 has been described as a biomarker of RUNX1 and FLI1 gene alterations. MPS didn't detect mutations in those genes although poor coverage was observed for FLI1 exons 3 and 9. Sanger sequencing detected a homozygous *FLI1* mutation in exon 9 (c.970 C>T p.Arg324Trp). Both parents were heterozygous for that mutation and consanguinity was discovered in previous generation of the family. EM showed organelle centralization, obscured microtubules, under developed OCS and abnormally large and electron dense α-granules.

Conclusion: In a Caucasian family, after the detection of increased MYH10 in platelets, we identified a homozygous *FLI1* mutation responsible for thrombocytopenia and severe platelet dysfunction. MYH10 platelet protein detection has proven to be useful in identifying the genetic abnormality in this family.

Disclosure of Interest: None declared.

OR003

A gain-of-function mutation in filamin-a potentiates platelet integrin αIIb/Beta-3 activation

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Background: Dominant mutations of the X-linked filamin A (*FLNA*) gene are responsible for filaminopathies A, rare disorders including brain periventricular nodular heterotopia (FLNA-PVNH), skeletal dysmorphism or congenital intestinal pseudo-obstruction (CIPO). We showed that in female PVNH patients, *FLNA* mutations lead to thrombocytopenia with giant platelets, exhibiting low adhesive and

secretory functions, correlating with low residual FLNa from the normal allele, the mutant FLNa being undetected.

Aims: We wish to discover new FLNa mutations leading to platelet defects.

Methods: Platelet functions (aggregation, secretion, adhesion) and platelet signaling induced by various agonists were investigated.

Results: The unique X-linked *FLNA* allele of a male patient with PVNH and CIPO, exhibited a stop codon mutation leading to an 100 amino acids-long FLNa C-terminal extension (NP_001447.2: p.Ter2648SerextTer101). Platelet counts were normal, with few enlarged platelets. Mutant FLNa was detectable in all platelets but at 30% of control level. Surprisingly, all platelet functions were significantly up-regulated: platelet aggregation and secretion, as induced by ADP, collagen, or VWF in the presence of ristocetin, as well as thrombus formation in blood flow on collagen or on VWF. Most importantly, ADP induced an increase in $\alpha_{IIb}\beta_3$ integrin activation contrasting with normal Rap1 activation, strongly suggesting mutant FLNa affected $\alpha_{IIb}\beta_3$ directly and not through signaling pathways upstream Rap1. Accordingly co-immunoprecipitation of FLNa with $\alpha_{IIb}\beta_3$ demonstrated a weaker interaction of mutant FLNa with β_3 in the patient's platelets, compared to control.

Conclusion: We postulate that the decrease in the association of mutant FLNa with β_3 , facilitates talin recruitment by β_3 , leading to increased $\alpha_{IIb}\beta_3$ activation and the ensuing gain-of-platelet functions observed in this patient's platelets.

Disclosure of Interest: None declared.

OR004

Histone-associated thrombocytopenia: a new cause of thrombocytopenia in critically ill patients

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Background: Thrombocytopenia is common in critically ill patients and is an independent predictor of prolonged intensive care unit (ICU) stay and mortality. However, the underlying causes remain largely unclear. More recently, extracellular histones could induce thrombocytopenia when injected into experimental mice.

Aims: Investigate the association between circulating histones and thrombocytopenia in ICU patients.

Methods: A prospective, longitudinal study of ICU patients with thrombocytopenia as defined by platelets (PLTs) $< 150 \times 10^9/L$ and/or $\geq 25\%$ PLT drop within first 4 days of admission.

Results: Among 160 ICU patients, 47 (29%) had thrombocytopenia and their median histone levels were $\sim 24 \mu g mL^{-1}$. 7 of these 47 patients had no detectable histones with 6/7 surviving (mortality 14.2%). In the remaining 40 patients, median histone levels were $46.5 \mu g mL^{-1}$ and the mortality rate was higher at 37.5%. Patients with histones $\geq 30 \mu g mL^{-1}$ on admission had more significant PLT drops on 2nd (26.8% vs 0%, $P = 0.012$) and 3rd (35.9% vs 17%, $P = 0.005$) ICU days. There was a significant inverse linear correlation between histone levels on admission and PLT counts on 2nd ($r = -0.384$, $P = 0.009$) and 3rd ICU days ($r = -0.402$, $P = 0.006$). In addition, treating freshly isolated human PLTs with these patients' plasma caused significant PLT aggregation, which was abrogated by anti-histone antibodies. Also, 29 of these 40 patients had disseminated intravascular coagulation (DIC) by ISTH criteria with 19/29 (65.5%) having histone $\geq 30 \mu g mL^{-1}$ to suggest a link between very high histone levels and DIC ($P = 0.0016$). However, 11/40 patients had histone-associated thrombocytopenia without DIC or other known causes and 28 day-mortality remained high in this group at 36.3%.

Conclusion: Histone-associated thrombocytopenia is a new cause of thrombocytopenia that is associated with adverse clinical outcomes in critical illness. Novel translational strategies to detect and neutralise cytotoxic circulating histones are therefore exigent.

Disclosure of Interest: None declared.

OR005

Platelet mitochondrial dysfunction in patients with sepsis or cardiogenic shock

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Background: The degree of platelet mitochondrial alterations during human sepsis or cardiogenic shock is not well established, as it is not studied if such alterations can influence platelet responsiveness to aggregating agents.

Aims: Determining platelet mitochondrial alterations in septic shock and establishing correlation with platelet function tests

Methods: A prospective, observational case-control clinical study on sixteen patients affected with severe sepsis or septic shock and sixteen patients with cardiogenic shock enrolled within 48 h from admission to intensive care unit. Platelet mitochondrial biochemistry was studied with spectrophotometry at 30 °C and results were expressed relative to citrate synthase activity, a marker of mitochondrial density. Platelet function was studied by lumi-aggregometry and dense granules content measurement.

Results: platelets of patients with sepsis or cardiogenic shock similarly showed to have a lower activity of mitochondrial respiratory chain nicotinamide adenine dinucleotide dehydrogenase (NADH) ($P < 0.001$), NADH-ubiquinone 1 reductase (complex I) ($P = 0.006$), NADH-cytochrome c reductase (complex I and III) ($P < 0.001$) and cytochrome c oxidase (complex IV) ($P < 0.001$) activities than controls. Platelets of patients with sepsis were hyporesponsive to adenosine diphosphate, collagen, U46619 and thrombin receptor activating peptide compared to that of controls, both in terms of maximal aggregation ($P < 0.001$ for each agonist) and of secretion ($P < 0.05$ for each agonist). Primary wave in response to ADP diminished in parallel with mitochondrial NADH (R2 0.36; $P < 0.001$), complex I (R2 0.38; $P < 0.001$), complex I and III (R2 0.27; $P = 0.002$) and complex IV (R2 0.43; $P < 0.001$).

Conclusion: Platelet mitochondria are altered during human sepsis and cardiogenic shock. In platelets of patients with sepsis, mitochondrial dysfunction is associated with general hyporesponsiveness to aggregating agents.

Disclosure of Interest: None declared.

Atherosclerosis

OR006

Swapping over two peptide epitopes derived from APOB and C5aR located at terminuses of recombinant proteins maintains the similar effective reduction on atherosclerotic lesion in B6;129S-LDLR(TM1HER) APOB(TM2SGY)/J mice

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Background: Atherosclerosis is increasingly recognized as a complex chronic inflammatory disease. Vaccination against atherosclerosis by using epitopes from LDL Apolipoprotein B (ApoB) protein, heat shock protein (HSP) and from complement component 5a receptor (C5aR) limited neointimal hyperplasia and inflammatory cell content.

Aims: To assess whether two terminuses of a recombinant protein can be modulated by swapping over two peptide epitopes derived from ApoB and C5aR protein located at different terminuses of the protein through immunizing B6;129S-Ldlr^{tm1Her} ApoB^{tm2Sgy}/J mice for their effective reduction on atherosclerotic lesions.

Methods: Antigenic epitopes derived from human ApoB (AA688-707) designated as epitope A, human HSP60 (AA516-528) as epitope H^b, mycobacterium HSP60 (AA253-268) as H^m and C5aR (AA1-31) as R were incorporated into a dendroaspilin scaffold to generate AH^bH^mR and RH^bH^mA, containing same epitopes, but with a different epitope at terminuses of the molecules.

Results: Histological analyses demonstrated that the mice immunized with AH^bH^mR and RH^bH^mA showed equally significant reductions in the size of atherosclerotic lesions (70.2% and 65.9%; $P < 0.001$, respectively) compared with controls. These results were in agreement with those in descending aortas showing significant decrease in lesion area (50% for AH^bH^mR and 42% for RH^bH^mA, respectively, $P < 0.001$). This effect on lesion reduction was correlated with the cellular responses towards decreased inflammatory cell and increased regulatory T-cell contents at local (lesion sites) and remote areas (splenocytes).

Conclusion: Immunization of mice with these two constructs containing multiple epitopes effectively reduced early atherosclerotic lesions. Two terminuses of the recombinant molecules are flexible for antigenic effect after introduction either ApoB peptide or C5aR peptide. This approach offers attractive opportunities for the design of protein-based, multivalent vaccines against atherosclerosis.

Disclosure of Interest: None declared.

OR007

Examining the hexosamine biosynthesis pathway in hyperglycemia-induced accelerated atherosclerosis

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Background: Cardiovascular disease (CVD) is responsible for the majority of deaths in people with diabetes. Our lack of understanding of the biochemical mechanisms linking diabetes and CVD complicates the development of effective treatment strategies. Elevated flux through the hexosamine biosynthesis pathway (HBP) is a notable intracellular effect of hyperglycemia that is associated with the induc-

tion of endoplasmic reticulum (ER) stress. ER stress has been implicated in the development of accelerated atherosclerosis.

Aims: This study investigated the role of enhanced HBP flux and ER stress on atherogenesis in hyperglycemic ApoE^{-/-} mice.

Methods: Hyperglycemia was induced in ApoE^{-/-} mice by introducing a point mutation (C96Y) in one copy of the insulin 2 gene. Immunofluorescent imaging was used to quantify the level of ER stress markers (CHOP and GRP78), O-linked glycosylation (O-GlcNAc) and the expression of glutamine fructose-6-phosphate amidotransferase (GFAT, the rate-limiting enzyme of the HBP). MALDI-imaging was used to measure the end product of the HBP, uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc).

Results: ApoE^{-/-}Ins2^{+/-}Akita mice develop both hyperglycemia (FBG = 15.0 ± 1.1 vs. 7.4 ± 0.3 mM in ApoE^{-/-} mice) and accelerated atherosclerosis (3.4 fold increase in lesion area, $P < 0.01$) relative to ApoE^{-/-} controls. Hyperglycemic mice showed ~2-fold increase in the levels of vascular ER stress markers ($P < 0.01$) and O-GlcNAc ($P < 0.05$). Enhanced expression of GFAT, UDP-GlcNAc and O-GlcNAc ($P < 0.01$) were also observed in hepatic tissues. These results are consistent with an elevated flux through the HBP in hyperglycemic mice. Treatment with the chemical chaperone 4-phenylbutyric acid significantly reduced ER stress levels ($P < 0.05$) and attenuated accelerated lesion development in hyperglycemic mice ($P < 0.05$).

Conclusion: These findings support the role of the HBP in hyperglycemia-induced accelerated atherosclerosis.

Disclosure of Interest: None declared.

OR008

Tolerance to multiantigenic molecule expressing peptides from APOB100, HSP60 and outer membrane protein of chlamydia pneumonia reduces markers of plaque instability and stabilizes advanced atherosclerosis in APOB TM2SGY/LDLRTM1HER/J mice

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Background: Immune modulation is gaining acceptance as an effective therapy to attenuate disease pathology in atherosclerosis. The challenges of immune therapy are to identify specific antigens to activate an atheroprotective immune response at different stages of disease and to stabilize an unstable plaque. We have earlier shown that a oral tolerance to recombinant molecule expressing three peptides derived from ApoB100, human HSP60 (hHSP60) and outer membrane protein (OMP) of *Chlamydia pneumonia* (Cpn) (AHC) offers protection against development of atherosclerosis in mice

Aims: To explore the effect of tolerance to three atherogenic peptides expressed in AHC molecule in controlling advanced atherosclerosis and inducing plaque stabilization in ApoB^{tm2Sgy}/Ldlr^{tm1Her}/J mice.

Methods: ApoB^{tm2Sgy}/Ldlr^{tm1Her}/J mice were fed a high-fat diet for 10 weeks, to establish lesion, orally dosed with AHC molecule or ovalbumin as control in the last 1.5 weeks, and continued this diet for the next 12 weeks. Animals were sacrificed after 22 weeks HFD for the assessment of plaque composition

Results: Histological analysis showed a comparable increase in lesion area in the aortic sinus in both groups but the necrotic area reduced by 39.2% ($P = 0.001$) in AHC treated animals (16.5 ± 1.2 vs. 10.5 ± 0.5). We observed significant reduction in markers associated with plaque instability in AHC treated animals. Expression of MMP9 ($P = 0.006$), tissue factor ($P = 0.001$) and plaque apoptosis ($P = 0.035$) were reduced while collagen content ($P = 0.002$) was sig-

nificantly higher in AHC treated mice suggesting features of plaque stabilization in these animals. Plaque stabilization was associated with to decrease in adaptive immune response

Conclusion: Our results suggest that oral tolerance to three atherogenic peptides derived from ApoB, HSP60 and Cpn outer membrane protein can induce markers of stabilization in mice with advanced atherosclerosis. These results offer a novel therapeutic option for the stabilization of a vulnerable plaque.

Disclosure of Interest: None declared.

OR009

Modulation of clinically relevant pathways by post translation modification regulatory system in coronary artery disease

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Background: Coronary artery disease (CAD) is one of the endemic diseases causing enormous social and economic loss however much needed understanding of modulation of molecular pathways leading to the disease are less understood.

Aims: In this study we tried to evaluate the post translational modification (PTM) regulatory mechanisms modulating the CAD associated pathways and leading to identification of potential biomarkers and drug targets.

Methods: CAD associated genes were extracted using manual curation and most comprehensive datamining tools from clinical trials database (clinicaltrials.gov), Uniprot, DrugBank, Ensembl, HPRD, CADgene database MEDLINE and PUBMED. Further we have identified distribution of PTMs for CAD genes and the whole genome using Perl programming. Using PTMCODE2 database we have identified significantly coevolving PTMs and constructed network of the same using Cytoscape 3.0.1 and prioritized the PTMs based on network topological parameters "degree distribution and betweenness centrality". Using PhosphoPOINT database and based on frequency of specific PTM inducing proteins we have identified potential biomarkers/ drug targets.

Results: We have identified 943 genes associated with CAD which had a similar distribution of PTMs to that of whole genome. Further construction of network of PTMs suggested that serine phosphorylation, threonine phosphorylation, acetylation and n-glycosylation are important regulatory PTMs. Based on the enzymes which modulate the network of above mentioned PTMs, we found 5 most important kinases such as PRKCA, GSK3B, PRKCD, SRC and PRKACA to regulate clinically relevant pathways associated to CAD.

Conclusion: We have identified 4 potential PTMs and the enzymes which regulate these PTMs in CAD. Further analysis of these kinases may give important information regarding perturbation of these pathways in the disease onset and progression.

Disclosure of Interest: None declared.

OR010

Gene centric analysis combined with network analysis to identify genes associated with coronary artery disease

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Background: Coronary artery disease (CAD) is complex, influenced by both genetic and environmental factors and causes perturbations across multiple organ systems.

Aims: We prioritized genes for CAD using pathway and tissue specific interaction network, supported by experimental validation in a case-control cohort.

Methods: We integrated the data obtained from *CARDIoGRAMplusC4D* consortium and global gene expression data sets derived from *GEO* to identify genes associated with CAD. The prioritized genes were then assigned pathways using *ClueGO*. Overlapping pathways were used to construct *Pathway Interaction Network (PIN)*, ranked based on *Jaccard index* and visualised using *Gephi*. Genes within top ranked pathways were used to construct the *Protein-Protein Interaction (PPI)* network. Further, a tissue specific PPI network was constructed to understand the interaction of these genes in different tissues that are involved in the disease process. Expression of key genes was experimentally validated in 50 CAD subjects and 50 controls.

Results: Analysis of GWAS and GEO data yielded 509 and 1459 genes and belonged to 232 and 699 pathways, respectively. Top 23 pathways having weighted degree > 4 included 208 genes that were used to construct a PPI network. Network analysis using topological parameters like weighted degree, node degree and betweenness centrality helped in identifying 16 key candidate genes (JUN, SMAD3, SMAD4, PTPN11, VEGFA, FOS, VIM, EGF, FN1, SOS1, JAK1, MET, CDC42, CDKN1A, MMP9 and NCK1). Tissue specific network analysis showed that 80% of the key genes were expressed across all tissues, signifying their contribution to CAD development. There was significant differential expression of these candidate genes in cases than in the controls.

Conclusion: Pathway based interaction network is effective in identifying key genes for CAD and sheds light on their interaction across various tissues involved in the disease process.

Disclosure of Interest: None declared.

Hemophilia – clinical

OR011

Leopold trial results: correlation of von willebrand factor antigen level with bay 81-8973 pharmacokinetic parameters of patients with severe hemophilia A

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Background: Increasing evidence suggests that endogenous von Willebrand factor antigen (vWF:Ag) levels have a significant effect on the pharmacokinetics (PK) of factor VIII (FVIII) in patients with hemophilia A.

Aims: To evaluate the correlation of vWF:Ag level with FVIII PK parameters in patients with severe hemophilia A receiving BAY 81-8973, Bayer's new full-length recombinant FVIII

Methods: Analyses were performed using data from the open-label, randomized LEOPOLD I and II trials, which evaluated PK, efficacy, and safety of BAY 81-8973 in patients with severe hemophilia A. BAY 81-8973 PK parameters were measured using the FVIII one-stage clotting and chromogenic assays. Spearman rank correlations were used to study the association between vWF:Ag level and BAY 81-8973 PK parameters.

Results: A total of 28 patients (mean age, 31.6 years) from LEOPOLD I (n = 25) and LEOPOLD II (n = 3) were evaluable for BAY 81-8973 PK correlation analyses. The median vWF level was 103.5%. A cut-off of 120% for high vWF was used similar to a previous study of Lalezari et al (*Haemophilia*. 2014;20:e15-22). Geometric mean half-life

was 1.5-fold higher in those with vWF:Ag levels $\geq 120\%$ (17.9 h; $n = 9$) vs $< 120\%$ (11.8 h; $n = 19$). Because the results were similar for both assays, only one-stage assay results are reported here. vWF:Ag level positively correlated with clearance-dependent BAY 81-8973 PK parameters, including area under the curve ($r = 0.73$, $P < 0.0001$), half-life ($r = 0.77$, $P < 0.0001$), mean residence time ($r = 0.80$, $P < 0.0001$), and 48-h trough level ($r = 0.81$, $P < 0.0001$). vWF:Ag level correlated negatively with BAY 81-8973 clearance ($r = -0.79$, $P < 0.0001$). No significant correlation was observed with maximum FVIII concentration.

Conclusion: BAY 81-8973 PK parameters significantly correlate with vWF:Ag levels in patients with severe hemophilia A. High vWF levels increased the half-life of BAY 81-8973 in circulation 1.5-fold compared with low vWF levels.

Disclosure of Interest: S. Lalezari Speaker Bureau of: Has been a speaker for Bayer and has been reimbursed by Bayer and Pfizer for meeting attendance, T. Fujii: None declared, Y. Kwong: None declared, H. Delesen Employee of: Bayer Pharma AG, A. Shah Employee of: Bayer HealthCare, C. Tückmantel Employee of: Bayer Pharma AG, W. van Heerde Consultant for: Bayer, M. Maas Enriquez Employee of: Bayer Pharma AG.

OR012

In haemophilic patients tfpi levels influence thrombin generation profile and TFPI neutralization normalizes thrombin generation potential

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Background: TFPI is a potent direct inhibitor of factor Xa, inhibiting the factor VIIa/TF complex in a factor Xa-dependent manner. TFPI feedback inhibition of factor VIIa/TF is consistent with deficient factor Xa production in hemophilic plasma.

Aims: In this multicenter study, we evaluated the effect of TFPI on thrombin generation (TG) parameters and the impact of TFPI neutralization on normalization of TG.

Methods: 101 hemophilic (H) patients (65 HA and 36 HB) were included. TG was evaluated using the CAT system (Hemker, 2002) in platelet-poor plasma (PPP) and platelet-rich plasma (PRP) at low tissue factor (TF) concentration (1 pM) before and after adding anti-human TFPI antibody (A-TFPI Ab, R & D System) at 1 $\mu\text{g.mL}^{-1}$ final concentration. Blood was withdrawn into citrated Monovette tubes (Sarstedt) containing corn trypsin inhibitor (CTI). Free TFPI (fTFPI) levels were measured by ELISA (Stago). Correlations were evaluated according to Spearman's R^2 correlation coefficient and were considered significant at $P < 0.05$ (*).

Results: In PPP, TG parameters endogenous thrombin potential (ETP), thrombin peak (P) and velocity index (VI) were inversely correlated with fTFPI level irrespective of hemophilia severity. For ETP, P and VI, R^2 was respectively 0.299*, 0.312* and 0.318*. fTFPI levels ranged from 5.3 to 26.5 ng.mL^{-1} (Q1-Q3: 9.3–12.9). At fTFPI levels above 12.9 ng.mL^{-1} , ETP and P were significantly lower than at levels under 12.9. In PRP, LT was correlated with fTFPI irrespective of hemophilia severity ($R^2 = 0.160$ *). This correlation was much stronger for severe HA ($R^2 = 0.407$ *). In patients with severe HA, both ETP

and P were inversely correlated with fTFPI ($R^2 = 0.200$ * and $R^2 = 0.247$ * respectively). A-TFPI Ab reduced LT by 65% in PRP. Moreover, blocking TFPI by A-TFPI Ab led to complete normalization of TG profile both in PRP and in PPP, irrespective of hemophilia severity.

Conclusion: fTFPI greatly influences TG potential in hemophilic patients.

Disclosure of Interest: None declared.

OR013

DDAVP in moderate hemophilia a patients: a treatment strategy worth considering

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Background: DDAVP increases factor VIII activity (FVIII:C) in patients with nonsevere hemophilia A (HA). However, there is a large inter-individual variation in the response to DDAVP. A peak FVIII:C > 30 IU dL⁻¹ is considered to be adequate for treatment in case of minor bleeding. Patients with a lower baseline FVIII:C show reduced response rates compared to patients with a higher baseline FVIII:C. Moderate HA patients (baseline FVIII:C 2–5 IU dL⁻¹) might therefore be less frequently tested for or treated with DDAVP. As DDAVP is an important therapeutic modality in HA we evaluated the response in moderate HA patients.

Aims: To describe the response to DDAVP in moderate HA patients.

Methods: The RISE study is an international cohort study including data of 1210 nonsevere HA patients with known DDAVP response that received DDAVP between 1980 and 2012. Patients were defined as moderate if their baseline FVIII:C on the day of DDAVP administration was 2–5 IU dL⁻¹. Main outcome is the response to DDAVP, based on the highest measured FVIII:C after administration, defined as: complete (CR; FVIII:C > 50 IU dL⁻¹), partial (PR; FVIII:C 30–50 IU dL⁻¹) or no response (none of the above).

Results: In this analysis we included all 81 patients with moderate HA among the patients of the RISE study. Median baseline FVIII:C was 4 IU dL⁻¹ (IQR 3–5). In the total group the median peak FVIII:C after DDAVP administration was 18 IU dL⁻¹ (IQR 9–26). CR occurred in 2 patients (2%), peak FVIII:C was 57 IU dL⁻¹ and 51 IU dL⁻¹ respectively. Fifteen patients (19%) showed a PR, median peak FVIII was 35 IU dL⁻¹ (IQR 32–42) while all other patients ($n = 64$, 79%) did not show a response.

Conclusion: In 21% of the patients in this European/Australian/Canadian cohort of moderate hemophilia A patients, the DDAVP response was adequate for treatment in case of minor bleeding or trauma.

Therefore, it is important to perform a DDAVP test in patients with moderate hemophilia A.

Disclosure of Interest: None declared.

OR014

The impact of patient adherence on FVIII levels: a comparison of rFVIII and rFVIII-Fc regimens

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Background: Because adherence is a multi-factorial behavior, it is unknown how fewer infusions may or may not affect it. Therefore, it is important to understand the impact of adherence to rFVIII and rFVIII-Fc regimens on FVIII levels.

Aims: To evaluate time above and below specific FVIII levels for patients missing 1 or 2 doses per month for rFVIII and rFVIII-Fc prophylactic regimens.

Methods: A literature-based model was developed. Factor levels were estimated using a one-compartment pharmacokinetic model (Collins 2010). Half-life and incremental recovery were taken from a crossover study of rFVIII and rFVIII-Fc (Young 2013). The following prophylaxis regimens were evaluated: 35 IU kg⁻¹ rFVIII, 3x/week; 50 IU kg⁻¹ rFVIII-Fc, Q4D; and 50 IU kg⁻¹ rFVIII-Fc, Q5D. The model ran for 1 month and simulated the impact of missing one or two doses per month on each regimen. Each scenario modeled was compared on the following metrics: time below 1% and 3% and time above 10% and 20% for the average week.

Results: A patient missing one or two doses on rFVIII was estimated to have 11.6 and 23.2 fewer hours per week below 1% and 2.9 and 17 h fewer hours per week below 3% compared to missing one or two doses on rFVIII-Fc Q4D. This difference increased to 23.6 and 47.2 fewer hours below 1% and 30.1 and 46.1 fewer hours below 3% for a patient missing one or two doses on rFVIII-Fc Q5D. A patient would also have 7.3 and 14.3 more hours above 10% and 1.7 and 7 more hours above 20% factor levels when missing one or two doses per month on rFVIII compared to rFVIII-Fc dosed Q4D. This increased to 24 and 31 more hours above 10% and 13.6 and 19 more hours above 20% for a patient missing one or two doses on rFVIII-Fc Q5D.

Conclusion: This model suggests that a patient who is not fully adherent on rFVIII may have less FVIII protection on rFVIII-Fc dosed every four or five days. Physicians should consider this information before prescribing rFVIII-Fc for a patient who is not fully adherent.

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OR015

Functional effects of F8 missense mutations on DDAVP response in nonsevere hemophilia A brothers

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Background: Large inter-individual variation in DDAVP response is observed in nonsevere hemophilia A patients. Further analysis of known determinants of the response may support understanding of the exact mode of action and optimal clinical use of DDAVP. F8 mutation may influence the response to DDAVP in nonsevere hemophilia A patients.

Aims: To evaluate whether the functional effects of F8 missense mutations influence the response to DDAVP.

Methods: The RISE study is an international cohort study including data of 1210 nonsevere hemophilia A patients from Europe, Canada and Australia with known DDAVP test results. We defined absolute response to DDAVP as the difference between baseline factor VIII (FVIII:C) and the peak level of FVIII:C following DDAVP administration. Missense mutations are classified according to the domains of the FVIII protein (respectively A1, A2, A3, B, C1 and C2) in which they are located. We compared the association between genotype and absolute response to DDAVP using linear regression with Generalized Estimating Equations (GEE) to correct for the relatedness between brothers.

Results: For this analysis, 76 patients from 37 families with known F8 missense mutations were included. They displayed 24 different missense mutations. Median FVIII:C before and after DDAVP was respectively 18 (IQR 10–25) and 57 (IQR 39–88) IU dL⁻¹. Missense mutations in the A1, A2, A3 and B domain were associated with an absolute increase in FVIII:C upon DDAVP administration ($P \leq 0.002$). Missense mutations in the C1 and C2 domains were not associated with an absolute increase in FVIII:C following DDAVP ($P > 0.05$). As the functional effects of mutations in the C1 and C2 domain of the F8 gene mainly involve von Willebrand factor binding this may influence DDAVP response.

Conclusion: Defects in the C1 and C2 domain of the F8 gene associate with a lack of DDAVP response. This may be due to reduction in factor VIII binding to von Willebrand factor, caused by the underlying mutation.

Disclosure of Interest: None declared.

Anti-platelets – basic

OR016

Ticagrelor and clopidogrel attenuate the prothrombotic state induced by bacterial endotoxemia

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Background: In the PLATO study, the novel platelet P2Y₁₂ inhibitor ticagrelor was unexpectedly associated with fewer deaths following pulmonary infections and sepsis than clopidogrel.

Aims: To determine whether greater P2Y₁₂ inhibition by ticagrelor compared to clopidogrel would result in greater reduction in platelet-leukocyte interactions, thereby reducing inflammatory responses and the prothrombotic state associated with systemic inflammation.

Methods: Thirty healthy volunteers were randomized to ticagrelor 90 mg bd (*n* = 10), clopidogrel 75 mg od (*n* = 10) or no antiplatelet medications (controls; *n* = 10) for one week. *E. coli* endotoxin (LPS) 2 ng kg⁻¹ was then administered intravenously. Plasma fibrin clot structure was assessed *ex vivo* using a validated turbidimetric assay and D-dimer was measured using the INNOVANCE assay. Informed consent was obtained. The study was approved by a medical ethics committee and funded by the Medical Research Council (UK).

Results: In the control group, fibrin clot maximum absorbance (an indicator of fibrin clot density), increased by 109 ± 37% at 6 h after LPS administration compared to baseline (*P* < 0.001). The percentage increase in maximum absorbance was significantly lower in the ticagrelor group (33 ± 19%; *P* = 0.02) compared to control, but was not significantly lower in the clopidogrel group (67 ± 20%; *P* = 0.20). In the control group, plasma levels of the fibrin degradation product D-dimer increased from 192 ± 36 µg L⁻¹ at baseline to a peak of 2217 ± 447 µg L⁻¹ at 4 h after LPS (*P* < 0.001). Peak levels of D-dimer were 48% lower in the ticagrelor group (*P* < 0.001) and 19% lower in the clopidogrel group (*P* = 0.01) compared to control.

Conclusion: To our knowledge, this is the first study to show that ticagrelor reduces prothrombotic changes in fibrin clot structure induced by bacterial endotoxaemia *in vivo*. The greater potency of the effects of ticagrelor compared to clopidogrel provides a potential mechanism for the reduced mortality following sepsis observed in the PLATO study.

Disclosure of Interest: None declared.

OR017

Platelet signaling and endothelial FC gamma receptor iib are both required for antibody-induced glycoprotein VI ectodomain shedding *in vivo*

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Background: The activatory collagen receptor glycoprotein (GP) VI contributes to normal hemostasis, but also to occlusive thrombus formation. The anti-GPVI antibody JAQ1 induces downregulation of the receptor in circulating platelets through ectodomain shedding accompanied by a transient thrombocytopenia *in vivo*. JAQ1-injection into

mice lacking LAT or PLCγ2 triggered GPVI-internalization without affecting platelet counts. Fc gamma receptors (FcγR) bind IgG antibodies and might thereby contribute to the *in vivo* effects of JAQ1. Mice bear three different activating FcγR (I, III and IV), which are expressed on different immune cells. FcγRIIb, the only inhibitory FcγR in mice, is present on various immune cells and some endothelial cells, but not on platelets.

Aims: We speculated that FcγRs on cells other than platelets might contribute to JAQ1-triggered effects *in vivo*.

Methods: We studied the *in vivo* effects of JAQ1 in mice pre-treated with the FcγRIIb/RIII blocking antibody 2.4G2 and in genetically modified mice.

Results: 2.4G2 pre-treatment or FcγRIIb-deficiency, but not the lack of FcγRIII, prevented JAQ1-induced thrombocytopenia and the appearance of soluble GPVI (sGPVI) in plasma. Histological analyses revealed that the transient thrombocytopenia was caused by FcγRIIb-dependent platelet sequestration in the liver. Kupffer cell depletion did not prevent the JAQ1-induced effects, indicating a contribution of endothelial cells in this process. To assess the role of platelet GPVI signaling, mice lacking Syk only in platelets were challenged with JAQ1. In the absence of Syk no sGPVI was detectable in plasma and the transient thrombocytopenia was abolished.

Conclusion: Our data demonstrate that anti-GPVI antibody triggered transient thrombocytopenia and the release of sGPVI from circulating platelets depend on 1) platelet GPVI signaling and 2) endothelial FcγRIIb.

Disclosure of Interest: None declared.

OR018

Small molecule DCDBS84 regulates platelet thrombus formation by targeting C-SRC SH3 domain

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Background: Selective inhibition of outside-in signaling is achieved by disrupting the interaction of integrin β3 cytoplasmic tail with c-Src by the RGT peptide through a binding site lying in the “N”-Src loop of the SH3 domain. However, *in vivo* experimental data are needed to assess the applicability of this strategy in antithrombotic therapy.

Aims: The present study aims to design and evaluate the small molecules mimicking the active structure of the RGT peptide that can selectively inhibit outside-in signaling-regulated platelet functions by targeting c-Src and finally enable the *in vivo* experiments.

Methods: The small molecule DCDBS84 was selected by means of the structure-based *in silico* screening approaches. The affinity of DCDBS84 binding to the c-Src SH3 was measured by SPR, and its binding site was mapped by NMR. Immunoprecipitation and pull-down assays were employed to determine whether DCDBS84 disrupts the β3/c-Src interaction. The effect of DCDBS84 on the typical platelet outside-in and inside-out signaling events was also tested. The *in vivo* thrombotic and hemostatic potential in the presence of DCDBS84 was evaluated in mice using the FeCl₃-induced artery thrombosis model and tail transection.

Results: DCDBS84 bound to the “N”-Src loop of the c-Src SH3 domain in NMR, with a K_d value at approximately 980 nM. This small molecule unequivocally disrupted the β3/c-Src interaction both in immunoprecipitation and pull-down assays. It also dose-dependently caused an inhibitory effect on platelet functions mediated by outside-in signaling, but not those by inside-out signaling. Of note, DCDBS84 could effectively suppress occlusive thrombus formation

after artery injury by FeCl₃ without affecting bleeding time and blood loss.

Conclusion: The present work provides the first *in vivo* evidence that targeting the c-Src SH3 domain results in a phenotype that substantially compromises in thrombotic potential without bleeding diathesis and may thus represent a rational choice for the future antithrombotic therapy.

Disclosure of Interest: None declared.

OR019

Congenital severe deficiency or pharmacological inhibition of the platelet P2Y₁₂ receptor for adenosine diphosphate does not impair the capacity of platelet to synthesize thromboxane A₂

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Background: Patients with acute coronary syndromes (ACS) are treated with acetylsalicylic acid (ASA) and antagonists of the P2Y₁₂ receptor (P2Y₁₂R) for adenosine diphosphate. It was surmised that they might be treated with P2Y₁₂R-antagonists only, based on the demonstration that these drugs inhibit thromboxane-A₂ (TxA₂) production (target of ASA). However, this demonstration contrasts with the observation that platelets congenitally deficient of P2Y₁₂R normally synthesize TxA₂.

Aims: To test whether P2Y₁₂R-antagonists have off-target/indirect inhibitory effects on TxA₂ production.

Methods: Serum TxB₂ (TxA₂ metabolite immunoassay) levels were measured in 3 patients with congenital P2Y₁₂R deficiency and 7 healthy subjects (in presence/absence of P2Y₁₂R-antagonists), and in 20 patients treated with prasugrel (10 mg q.i.d.) for 14 days. Arachidonic acid (AA, 1 mM) and collagen (0.5–10 µg mL⁻¹) induced platelet aggregation (PA, light transmission aggregometry) and TxB₂ production were measured in platelet-rich plasma (PRP) of healthy subjects in presence of P2Y₁₂R-antagonists (alone or with ASA) or a P2Y₁R-antagonist (MRS2500).

Results: P2Y₁₂R-deficient patients and healthy subjects have similar serum TxB₂ levels, not decreased by P2Y₁₂R-antagonists *in vitro*. Prasugrel treated patients showed normal serum levels of TxB₂. AA- and collagen-induced PA and TxB₂ production in PRP were inhibited by P2Y₁₂R-antagonists. However, P2Y₁₂R-antagonists did not inhibit TxB₂ production when PA was prevented by avoiding the stirring of PRP in the aggregometer. MRS2500 had similar effects on PA and TxB₂ production as P2Y₁₂R-antagonists. ASA inhibited TxB₂ production more effectively than a P2Y₁₂R-antagonist; only the combination of ASA and a P2Y₁₂R-antagonist inhibited PA induced by 10 µg mL⁻¹ collagen.

Conclusion: Congenital deficiency or pharmacological inhibition of P2Y₁₂R does not affect the platelet capacity to synthesize TxA₂. There is no pharmacological evidence that ACS patients may be safely treated with P2Y₁₂R-antagonists without ASA.

Disclosure of Interest: None declared.

OR020

The P2Y₁₂ antagonist act-246475 causes less blood loss than ticagrelor at comparable antithrombotic efficacy in a rat thrombosis model

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Background: The P2Y₁₂ receptor is a validated target for prevention of major adverse vascular events in patients with acute coronary syndromes (ACS), as shown by clopidogrel which leads to irreversible receptor blockade. Reversible P2Y₁₂ receptor antagonists have the potential to further improve efficacy without increasing the bleeding risk. Such a profile is considered critical since a post-hoc analysis of several ACS trials revealed that major bleedings are associated with increased long-term mortality.

Aims: To compare two reversible P2Y₁₂ antagonists, ACT-246475 and ticagrelor, in a thrombosis model by determining antithrombotic efficacy and effect on surgical blood loss.

Methods: Blood flow velocity in the rat carotid artery after FeCl₃-induced thrombus formation was quantified using a Doppler flow probe. In parallel, blood loss after surgical wounding was quantified in the same rats.

Results: ACT-246475 and ticagrelor prevented FeCl₃-induced reduction of blood flow in a dose-dependent manner. At the highest doses tested both antagonists completely abolished thrombus formation in the carotid artery as evaluated by histology. At comparable antithrombotic efficacy, ACT-246475 caused about 3-times less surgical blood loss than ticagrelor. To explain this surprising difference between the two P2Y₁₂ antagonists, their effects on the regulation of vascular tone were compared. Ticagrelor, but not ACT-246475, induced vasorelaxation of pre-contracted rat femoral arteries and inhibited constriction induced by electrical field stimulation or by phenylephrine.

Conclusion: ACT-246475 showed a 3-times wider therapeutic window than ticagrelor in a rat thrombosis model. Both compounds are reversible P2Y₁₂ antagonists but clearly differ in their off-target effects. As a consequence, ACT-246475 at comparable antithrombotic efficacy causes clearly less blood loss than ticagrelor probably because it does not interfere with the regulation of vascular tone.

Disclosure of Interest: M. Rey Employee of: Actelion Pharmaceuticals Ltd., P. Hess Employee of: Actelion Pharmaceuticals Ltd., K. Morrison Employee of: Actelion Pharmaceuticals Ltd., E. Weber Employee of: Actelion Pharmaceuticals Ltd., M. Clozel Employee of: Actelion Pharmaceuticals Ltd., M. Riederer Employee of: Actelion Pharmaceuticals Ltd., B. Steiner Employee of: Actelion Pharmaceuticals Ltd.

VWD – classification, subtypes – I

OR021

Genotyping of VWD patients in the Netherlands: phenotype-genotype discrepancies and 27 novel VWF gene mutations

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Background: Genotyping von Willebrand factor (VWF) gene in von Willebrand disease (VWD) patients is not yet routinely performed in clinical practice, but may be indicated for improving diagnosis of VWD, determining subtype and for genetic counseling.

Aims: To identify VWF gene mutations in a large cohort of moderate and severe VWD patients in order to perform phenotype-genotype analysis.

Methods: VWF gene mutation analysis was previously performed in 199 of 804 patients with moderate and severe VWD (VWF levels ≤ 30 IU dL⁻¹) included in the nationwide "Willebrand in the Netherlands" (WIN) study. Mutation analysis was performed using Sanger sequencing of all 52 exons including exon-intron boundaries in 54 patients; 28 exons in 127 patients; a varying number of exons in 16 patients; and only exon 28 specific for type 2B VWD in 2 patients. Genotyping was performed as routine practice in some hemophilia treatment centers ($n = 143$), in case of diagnostic difficulty ($n = 42$) or for research purposes ($n = 14$). Genotypes were compared with patients' phenotypic classification using the current ISTH criteria. VWF:Ag, VWF:Act, VWF:CB, FVIII:C and multimers were determined in a central laboratory. Medical Ethics Committee approval and informed consent were obtained.

Results: Mutations were identified in 36/60 type 1 patients, 110/111 type 2 and 25/28 type 3 patients. We observed phenotype-genotype discrepancies for 10% of patients: 6 patients with a type 2 mutation had a VWF:Act/VWF:Ag ratio > 0.7 , 4 with type 2B mutations had normal ristocetin-induced platelet aggregation tests, and the R1205H "Vicenza" mutation was found in 5 phenotypic type 3 and 3 type 2A VWD patients. Of 75 mutations identified, 27 were novel: 4 in type 1, 11 in type 2, and 10 in type 3 patients. In 26 (22 type 1) patients no causative mutation was found.

Conclusion: We provide further insight in the molecular background of VWD and illustrate the difficulties of VWD classification using only phenotype. Genotyping VWF may improve diagnosis and classification of VWD.

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OR022

Case study of two consanguineous brothers with type 3 von willebrand disease (VWD), inhibitors and anaphylactoid reaction to VWF

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Background: Type 3 vWD is a rare bleeding disorder affecting only 0.3–0.5 per million persons. The development of alloantibodies against vWF is an infrequent but serious complication of treatment of vWD and can be complicated by severe or life-threatening anaphylactic/anaphylactoid reactions when reexposed to vWF.

Aims: To describe therapeutic strategies for bleed management, allergen tolerance, and inhibitor eradication.

Methods: Two sibs ages 9 and 5, born to first cousin parents, came to our center in 2012 for treatment of Type 3 VWD. Diagnosed overseas after an older sib died of bleeding, both sibs had > 15 infusions of Wilate before coming to the US and reported mild respiratory distress with infusions, treated with albuterol. Despite attempted prophylaxis first with Humate-P and then Wilate, bleeding symptoms persisted and more severe anaphylactoid symptoms to both vWF products developed for each boy.

Results: Hemostasis regimen of aminocaproic acid and rFVIIa was moderately effective. Pre-procedure platelet infusion was successfully used for the younger, with rationale of vWF stored in transfused platelet alpha granule protecting from the alloantibody. A desensitization protocol was successfully developed for each brother and immune tolerance therapy (ITI) was initiated with daily mycophenolate mofetil (MMF)/Wilate. Persistent high-titer inhibitor after 6 months prompted change to Rituximab. Genetic evaluation revealed a shared, homozygous deletion in VWF gene c.658_7887del (p.G220_L2629del). Exons 7-47 of the VWF gene could not be amplified, indicating homozygous deletion of a large portion of the VWF gene.

Conclusion: Bleed management in patients with inhibitors and anaphylactoid symptoms to the missing protein is challenging. Use of antifibrinolytic agents, platelets, and rFVIIa provides hemostasis.

Desensitization to vWF is possible. Regimens for inhibitor eradication in type 3 vWD are poorly described. Our attempt with MMF failed; response to Rituximab is pending.

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OR023

Abnormal angiogenesis in type 2A and 2B von willebrand disease (VWD): comparative studies of quantitative and qualitative VWD using blood outgrowth endothelial cells

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Background: Angiodysplasia of the gastrointestinal tract has been associated with VWD. Recently, von Willebrand factor (VWF) has been identified as a negative regulator of angiogenesis.

Aims: The current study explores the effect of VWF mutations on angiogenic behavior using blood outgrowth endothelial cells (BOEC) from patients with all types and subtypes of VWD.

Methods: Queen's University provided ethical approval and patient consent was obtained. BOEC were isolated from 5 healthy controls and 8 VWD patients, comprising two type 1 (C633R and D75_G178del mutations), one type 2A (2377fsX), one 2B (V1316M), one 2M (L1383P), one 2N (R854Q) and two type 3 (C633R and Q419X/S293 fs). BOEC were analyzed for angiopoietin-2 (Ang-2) secretion via ELISA ($n = 3$ each) and storage using confocal immunofluorescence (IF). To explore angiogenic potential, proliferation at 144 h and matrix protein adhesion was assessed ($n = 3$ each).

Results: Ang-2 secretion was increased significantly compared to controls (6 ng mL⁻¹) in type 2A (11 ng mL⁻¹, $P = 0.005$), type 2B (11 ng mL⁻¹, $P = 0.002$), one type 1 (16 ng mL⁻¹, $P < 0.0001$) and one type 3 BOEC (12 ng mL⁻¹, $P < 0.0001$). Confocal IF demonstrated intact Ang-2 storage in Weibel-Palade bodies in all cells except type 2B and 3 BOEC. Proliferation was increased in type 2A (52200 cells, $P < 0.05$) and 2B BOEC (55100 cells, $P < 0.05$) compared to the control mean (40900 cells). However, a reduction in cell number occurred in both type 1 BOEC (26600 and 25000 cells, $P < 0.05$). Adhesion to collagen did not differ between BOEC, but VWF binding was increased from controls (6% bound) in the type 2B (10% bound, $P < 0.05$), 2M (11% bound, $P < 0.05$), 2N (15% bound, $P = 0.0005$), both type 1 (18% and 11% bound, $P < 0.02$) and one type 3 (18% bound, $P < 0.0001$) BOEC.

Conclusion: Despite great variability in BOEC angiogenic behavior across and within VWD subtypes, types 2A and 2B BOEC were the most abnormal compared with the controls. This may explain the apparent increase in clinical prevalence of angiodysplasia in type 2A and 2B VWD patients.

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OR024

Type 2N von willebrand disease: one variant but more than one disease

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Background: Type 2N von Willebrand disease (VWD2N) is a qualitative variant defined by a markedly decreased binding of VWF to FVIII (VWF:FVIII) and a recessive inheritance.

Aims: To evaluate in a large cohort of patients (pts) identified as type 2N VWD by the French Reference center for VWD the relationship between phenotypic and molecular features and the clinical expression.

Methods: Pts with VWF:FVIII<0.5 were enrolled. Genotypic analyses were centralized. The clinical data including the Tosetto bleeding score (BS) were collected.

Results: 102 pts (88 families) were fully characterized. The F/M ratio is 1.7, the median (range) age is 38 (7–87) year, the BS is 5 (-2-27). *VWF* gene analysis showed that the p.R854Q mutation is present in 93 pts (91%) who are either homozygous (Hz) (A1; n:43) or compound heterozygous (CHz) for a silent allele (sa) (A2; 41) or for another type 2N mutation (A3; 9). The 9 last patients (B) are Hz for a non p.R854Q 2N mutation (4) or CHz for a non p.R854Q mutation and a sa (5). The FVIII:C differs significantly among A1, A2, A3 and B: 23 (12–45), 17 (7–35), 20 (4–36), 4 (2–7) % respectively. The BS increases gradually from 2 (-2–12) in A1, 5 (0–14) in A2, 6 (2–11) in A3, 14 (6–27) in B. These differences are statistically significant (except A2 vs A3). Taking into account all available data, it is possible to classify the 102 pts into 3 categories (i) A1 pts (42%) have only a moderate FVIII:C deficiency, few spontaneous bleeding symptoms, few therapeutic needs (42% were never treated) (ii) B pts (< 10%) have very low FVIII levels, severe bleeding symptoms, frequent needs for replacement therapy (iii) A2 and A3 pts (50%) constitute an intermediate category: compared to A1 they have slightly lower FVIII rates (associated in some cases with low VWF:RCO), more frequent bleeding symptoms and more therapeutic needs.

Conclusion: Type 2N VWD variant cannot be reduced to a single disease.

Disclosure of Interest: None declared.

OR025

Heterogeneity of von willebrand disease type 3 in a french cohort of 75 patients

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Background: von Willebrand disease type 3 (VWD3), inherited as an autosomal recessive trait, is due to an almost complete deficiency of von Willebrand factor (VWF).

Aims: The clinical, phenotypic and molecular features of VWD3 patients (pts) from the cohort of the French Reference center for VWD were analyzed to better consider the heterogeneity.

Methods: Clinical data including the bleeding score (BS) and phenotypic data (FVIII, VWF:Ag levels and VWF multimer analysis) were compared to whole coding sequence *VWF* gene analysis including large genomic rearrangements screening.

Results: 75 pts (gender 31M/44F) were diagnosed VWD3. We found 72 distinct mutations (65% new). In 9 pts (12%), only one mutant allele was characterized. We distinguished 2 groups. Group A included 61 pts (81%) exhibiting FVIII ≤ 5 UI dL⁻¹, undetectable VWF:Ag levels, absence of whole range of plasma multimers; the median age at diagnosis was 11 months (range birth-29 years); the median BS was 17 (range 2-34); 34 pts (55%) benefit of long term prophylaxis (LTP) and 4 (6.5%) developed an inhibitor. Group B included 14 pts (19%) exhibiting FVIII > 5 UI dL⁻¹ (median 12, range 6-20), detectable VWF:Ag levels (median 2.7 UI dL⁻¹, range 1-7), visible LMW multimers; the median age at diagnosis was 19 months (range 1-5 years); the median BS was 13 (range 8-28); no pt had LTP and none developed an inhibitor.

Conclusion: In this large cohort with a high frequency of novel mutations, the molecular pathogenesis was completely elucidated in 88% of pts, suggesting deep intronic mutations in 12%. In group B pts, tiny amounts of VWF:Ag leading to higher FVIII levels seem to induce a less severe bleeding profile and no risk of inhibitor; in this group, we noted that the mutation p.R273W (listed in the LOVD2 database as responsible for either VWD3 or recessive VWD2A) was detected at an homozygous or compound heterozygous state in 5/14 pts. These data underline the need for a large prospective study as 3WINTERS-IPS.

Disclosure of Interest: None declared.

Acquired disorders – treatment – I

OR026

Acquired von willebrand syndrome and response to desmopressin

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Background: Desmopressin (1-deamino-8-D-arginine vasopressin, DDAVP) is a synthetic analog of vasopressin that stimulates the endogenous release of von Willebrand factor (VWF) and factor VIII:C

(FVIII:C) from endothelial cells and platelets, increasing their levels in plasma. Its use is described to treat bleeding episodes in patients with Acquired Von Willebrand Syndrome (AVWS).

Aims: To describe the response of VWF and FVIII:C to DDAVP administration in patients with AVWS.

Methods: Case-series of 17 patients with AVWS who underwent a test administration of DDAVP.

FVIII:C and VWF levels were measured before DDAVP infusion and after 1 h, 2 h and 4 h. Response to DDAVP was defined as: complete (both FVIII:C and VWF:RCo 50 IU dL⁻¹ or higher); partial (FVIII:C or VWF:RCo lower than 50 IU dL⁻¹ but increased at least 3-fold) or absent.

Results: Sex: 7 males and 10 females. Median age at diagnosis was 54 years (range 22-74). Underlying disorders were present in 16/17 patients: 10 monoclonal gammopathy of uncertain significance (6 IgGk, 3 IgGλ, 1 double component), 1 Waldenstrom's macroglobulinemia, 1 chronic lymphatic leukemia, 3 essential thrombocytopenia, 1 inflammatory bowel disease. Response to DDAVP after 1 h was complete in 12 patients (70%), partial in 2 patients (12%) and absent in 3 patients (18%). After 4 h, response was complete in 4/13 patients (31%, data not available in 4 patients). DDAVP was used in 11 patients (65%) to prevent bleeding during minor surgery or to treat minor bleeding events. Other therapeutic interventions were used in 9 patients (FVIII/VWF concentrate in 4 patients, high dosage immunoglobulins in 1 patients, both in 4 patients).

Conclusion: Only a minority of patients with AVWS (31%) showed a complete and sustained response after DDAVP infusion (4 h). Nevertheless, DDAVP was used in the majority of patients to prevent bleeding in minor surgeries or to treat minor bleeding events.

Disclosure of Interest: None declared.

OR027

Activated prothrombin complex concentrate in acquired hemophilia A: an Italian registry-THE F.A.I.R. study

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Background: Bleeding is the main manifestation of acquired hemophilia (AH) and is often severe. First line treatment is based on the use of bypassing agents; activated prothrombin complex concentrate (aPCC, FEIBA) in particular showed high hemostatic efficacy, but the management of treatment has not completely been standardized.

Aims: Objective of this study is to evaluate the dosage, efficacy and safety of FEIBA in an Italian population of AH patients with acute bleeding.

Methods: The study is a retrospective prospective national registry begun in December 2012. The retrospective cases were examined over the five previous years, while the prospective study is still ongoing. All AH patients > 18 years treated with aPCC for a bleeding episode were included after obtaining informed consent.

Results: 13 centers are involved in the study and 8 of them entered at least one patient. Data on 25 retrospective patients and 15 prospective ones have been collected. Male patients were 20 (50%) whose median age at diagnosis was 74 years (IQR 28–88). AH was idiopathic in 52.5% of patients. 90 bleeding episodes requiring treatment were reported, mostly occurring into muscles (45.5%) and skin (27.8%). Of these, major bleeds were 55%. aPCC was administered in a median bolus dose of 73.0 ± 25.6 U kg⁻¹, with 12-h median dose frequency (IQR 8–48) and a total mean number of doses of 12.03 ± 3.4 . Treatment was continued for a mean period of 6 ± 4.9 days. FEIBA was considered effective in 83/90 episodes (92.2%) and was associated with antifibrinolytic in 11% of cases. No venous or arterial thrombotic events were reported; nine patients died (22.5%) during the period of observation: one because of uncontrolled bleeding, one of sepsis during immuno-suppressive therapy, two of malignancy, and the others due to reasons unrelated to AH.

Conclusion: The data from the registry confirm the efficacy and safety of APCC in bleeding management of AH patients. Further information on treatment schedule will be obtained with completeness of data.

Disclosure of Interest: None declared.

OR028

Perioperative management of bleeds with recombinant porcine FVIII in patients with acquired hemophilia A

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Background: Acquired hemophilia A (AHA) is a rare bleeding disorder caused by inhibition of human factor VIII (hFVIII) by auto-antibodies. Surgical procedures in AHA patients are often delayed until hFVIII inhibitors are suppressed. A recombinant FVIII, porcine sequence (rpFVIII, OBIZUR), less susceptible to inhibition by anti-hFVIII antibodies, offers a new option to manage bleeds in AHA.

Aims: The report evaluates the response to peri- and post-operative rpFVIII replacement therapy in AHA patients.

Methods: This phase 2/3 open-label clinical trial investigated the efficacy and safety of rpFVIII in the treatment of serious bleeds in 28 AHA subjects. Response to rpFVIII treatment at 24 h was the primary endpoint (e.g. effective, partially effective). Treatment began with a 200 U kg⁻¹ dose of rpFVIII; further doses were based on the subject's target FVIII levels, anti-pFVIII titer, and clinical status. Subjects' informed consent and appropriate ethics committee approvals were obtained.

Results: Recombinant pFVIII was applied perioperatively for 3 major surgical interventions (1 hemicolectomy, 1 endoscopic retrograde cholangiopancreatography, 1 emergency tracheotomy) and up to 10 days post-surgery for management of a limb compartment syndrome in 2 other patients. Immediately after the first dose, FVIII activity rose to clinically significant levels in all subjects (baseline anti-hFVIII titer: 17–80 BU); all had a positive response to treatment at 24 h post-infusion. Hemostasis was achieved in all subjects during rpFVIII treatment (dose: 300–3600 U kg⁻¹). One patient died of a re-bleed 10 days after rpFVIII treatment ended, while receiving another hemostatic agent. A 4 BU baseline anti-pFVIII titer in the subject undergoing a tracheot-

omy did not affect treatment success. After treatment, 2 subjects developed anti-pFVIII inhibitors *de novo*. No SAEs or thrombotic events related to rpFVIII treatment occurred.

Conclusion: Recombinant pFVIII was safe and effective for peri- and post-operative use in AHA patients undergoing surgery.

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OR029

Real world data on treatment with feiba in patients with clotting factor inhibitors

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Background: There is paucity of patients with FVIII or FIX treated and followed in clinical trials or observational studies.

Aims: To collect real world data on safety and effectiveness of APCC.

Methods: A prospective, non-interventional, post-authorization safety study (PASS) was carried out in patients with hemophilia A or B and inhibitors treated with FEIBA - for 12 ± 2 months.

Results: FEIBA was prescribed to 69 subjects (85.2%) with congenital hemophilia A, 2 (2.5%) with congenital hemophilia B, and 10 (12.3%) with acquired hemophilia A (AHA) at 40 sites in 10 countries in EU, US and Canada. Forty-five (55.6%) subjects had FEIBA prescribed at baseline for prophylaxis and 36 (44.4%) for on-demand treatment. Nine out of 180 adverse events were deemed product-related: only 3 of them were serious. One occurred in congenital hemophilia patients (hemarthrosis) and 2 in AHA patients: a catheter-related infection and a deep vein thrombosis in a patient treated concomitantly with FEIBA and rFVIIa. Three unrelated deaths were reported during the 12-month follow-up showing the still high incidence of mortality in patients with inhibitors. At the end of the study, the hemostatic effectiveness was rated by the physician as excellent or good in 91% of total subjects, with highest rates reported in subjects on regular prophylaxis (97%). Interestingly, this study provided information on the mode of administration of FEIBA: in particular, the mean infusion rate was higher (3.8 U kg min⁻¹, min-max range: 0.9 – 23.5 U kg min⁻¹) than that recommended in the Summary of Product Characteristics of FEIBA (2 U kg min⁻¹) without side effects.

Conclusion: This study highlights safety and effectiveness of FEIBA and the importance of real world data in assessing in these rare patients under routine clinical practice. Another non-interventional cohort study has been initiated to evaluate FEIBA use and outcomes in this population (FEIBA-GO).

Disclosure of Interest: C. Negrier Consultant for: Novo Nordisk, Baxter, Bayer, CSL Behring, LFB, and Pfizer. Speaker Bureau of: Novo Nordisk, Baxter, Bayer, CSL Behring, LFB, and Pfizer, S. Voisin: None declared, F. Baghaei: None declared, A. Novack: None declared, J. Doralt: None declared, R. Crea: None declared, A. Gringeri: None declared.

OR030

"Short-term prophylaxis" regimen with activated prothrombin complex concentrate in the prevention of bleeding relapse in acquired hemophilia

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Background: Acquired Hemophilia (AH) is characterized by bleeding episodes which may often prove fatal. Antihemorrhagic therapy consists in using bypassing agents - activated prothrombin complex (FEIBA) and recombinant FVIIa - until bleeding is stopped. Relapses of hemorrhage are reported in 25% of patients and no treatment protocols to prevent these recurrences have been recorded so far.

Aims: The aim of our study was to assess whether a prophylaxis regimen with FEIBA, following the acute phase treatment, could be effective in preventing hemorrhage relapse.

Methods: We retrospectively observed the management of hemorrhagic episodes in consecutive willing patients with AH admitted to the Padua University Hospital and Pavia University Hospital from 2008 to 2014. Some of them received APCC only during the acute phase of bleeding, while the others were maintained on a short-term prophylaxis regimen after the acute phase. We compared these two strategies in terms of safety and efficacy in preventing bleeding relapse.

Results: Eighteen AH patients (male 61.1%) with acute bleeding were evaluated. Mean age was 64.7 ± 16.4 years; 11 cases were treated with the use of FEIBA limited to the acute phase, while in 7 cases the acute phase treatment was followed by a "short-term prophylaxis" with aPCC (mean dose of 77.3 ± 33.7 U/kg-1 day for a mean period of 12.7 ± 5.7 days in case of major bleeds, and mean dose of 28.5 ± 9.3 U/kg-1 day for a mean period of 12.25 ± 10.7 days in minor episodes). Six relapses in patients treated with FEIBA only during the acute phase and no bleeds in patients treated with the "short-term prophylaxis" strategy were observed. A 5-fold increase in the risk of hemorrhagic recurrences in individuals who did not receive the prophylaxis was found (OR 5.73, IC 1.05–7.24). No thromboembolic complications or other adverse events were reported in either treatment.

Conclusion: A prophylaxis regimen with FEIBA was shown to be effective in reducing bleeding relapse in AH.

Disclosure of Interest: None declared.

Reproductive Issues

OR031

Pathology of placenta in fetal and neonatal immune thrombocytopenia: roles of TH17 immune responses, anti-platelet antibodies and angiogenic factors

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Background: Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a life threatening disease often leading to severe bleeding diathesis and/or miscarriage although the incidence of miscarriage has not been adequately studied. FNAIT is due to a maternal immune response against fetal platelet antigens. Antibodies and Th17 cytokines may target antigen positive trophoblasts and cause miscarriage, but this hypothesis has never been explored.

Aims: To investigate whether Th17 cytokines and platelet antibodies impaired trophoblast invasion and placental function.

Methods: $\beta 3$ integrin deficient female mice were immunized with wild-type (WT) platelets and bred with WT males. Placental vascularisation and function were investigated by micro-computerized tomography (CT) and echography. Angiogenic and Th17 cytokines, placental growth factor (PlGF) and fms-like tyrosine receptors (Flt-1) were detected by ELISA.

Results: Growth restriction and fetal loss only occurred in immunized mothers around embryo day E14.5. Placentae of affected fetuses had significantly reduced vascularization and materno-placental perfusion as demonstrated by ultrasound and biotin perfusion. CT scans also confirmed a shadowy development of placental sponge capillaries. Increased Th1 and Th17 responses were observed in immunized mice. These pro-inflammatory responses may contribute to trophoblast Flt-1 over-expression which decreases plasma PlGF/sFlt-1 ratio. E.14.5, the end of organogenesis is concomitant with trophoblast invasion into spiral arteries, the remodeling of which is critical for healthy pregnancy. Cytokeratin-7, a trophoblast marker was significantly decreased in the placentas of immunized mice, suggesting poor invasion. Interestingly, maternal intravenous immunoglobulin therapy ameliorated survival of FNAIT fetuses.

Conclusion: Th17 cytokines and $\beta 3$ integrin antibodies impaired placental pro-angiogenic signalling and function. Reduced trophoblast invasion may cause poor materno-placental perfusion and fetus loss in FNAIT.

Disclosure of Interest: None declared.

OR032

Characteristics of venous thromboembolism in 3072 French women using combined or progestin-only contraception

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Background: Information on the clinical and biological characteristics of contraceptive users who develop venous thromboembolism (VTE) is scarce. Whether the characteristics of women with a first documented VTE differ according to the type of hormonal contraceptives including progestin-only contraceptives (POC) remain to be determined.

Aims: Using data from COREVE cohort, we assessed the characteristics of women with a first documented VTE by hormonal contraceptives. We also estimated the number of preventable VTE.

Methods: Consecutive outpatients with documented VTE who consulted at our haemostasis Unit between 2000 and 2009 were enrolled in our study. Of the 3072 aged ≤ 45 years women, 30% ($n = 923$) were non-users, 68% ($n = 2079$) combined hormonal contraceptives (CHC) and 2% ($n = 70$) POC users.

Results: CHC users were younger (29.0 ± 7.2 mean $\pm$ SD) as compared to non-users (31.6 ± 7.1) $P < 0.0001$ or POC users (33.6 ± 7.6) $P < 0.0001$. Body mass index was higher in POC users (25.5 ± 5.8) as compared with CHC users (23.0 ± 4.4) $P < 0.0001$. No difference in VTE familial history between the three groups was observed. CHC or POC users experienced less proximal deep venous thrombosis as compared to non-users (respectively 21%, 18% and 37%; $P < 0.0001$). CHC users had significantly more FV Leiden mutation than non-users (respectively 17% and 13%; OR = 1.36 [1.09–1.70]). CHC users had also significantly more associated VTE-precipitating factors such as venous sclerotherapy, travel or plaster whereas surgery or bed rest were significantly increased in non-users. Women using cyproterone-containing CHC were significantly younger ($P < 0.0001$), leaner ($P = 0.0002$) and had more travel-related VTE ($P = 0.002$) as compared with other types of CHC. Finally, more than 12% of CHC users had inadequate prescription of pill because of known VTE risk factors.

Conclusion: CHC users with first VTE differ from non-users with respect to clinical and genetic background. Our results highlight the substantial number of preventable VTE in young women.

Disclosure of Interest: None declared.

OR033

Characterising fibrin monomer complex and D-dimer profiles in pregnancy

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Background: Serum D-Dimers (DD) have a high negative predictive value for excluding venous thromboembolism (VTE) outside of pregnancy but the use in pregnancy remains controversial. A higher cut-off value has been proposed in pregnancy. Fibrin monomer complexes (FM) have been considered as an alternative diagnostic tool for exclusion of VTE in pregnancy due to its different behaviour.

Aims: To establish normal ranges of maternal serum levels of DD and FM in pregnancy and determine maternal characteristics and obstetric factors that affect them.

Methods: This was a prospective study in 4464 singleton pregnancies between 6 and 33 weeks of gestation. Serum DD and FM was measured by STA[®]-Liatest[®] FM (Diagnostica Stago) and STA[®]-Liatest[®] D-Di (Diagnostica Stago) assays on the STA-Compact[®] coagulation analyser by quantitative immunoturbidimetry. Multiple regression analysis was used to determine maternal characteristics and obstetric factors affecting measured DD and FM levels and the regression model was used to convert each measurement into a multiple of the median (MoM).

Results: Serum DD increased with gestational age and maternal weight and were decreased in cigarette smokers. DD were higher in sickle cell carriers and in women of African, Oriental and Asian racial origin compared to Caucasians. Serum FM also increased with maternal weight and gestational age and was affected by cigarette smoking and a positive medical history.

Conclusion: This study provides normal ranges for DD and FM in pregnancy and defines the maternal and pregnancy characteristics which affect these coagulation parameters. Maternal serum DD and FM concentrations reflect the hypercoagulable state in pregnancy and should be adjusted by maternal and obstetric characteristics to improve the utility of DD and FM as a diagnostic tool for VTE in pregnancy

Disclosure of Interest: None declared.

OR034

Pre-pregnancy BMI and delivery BMI as risk factors for postpartum VTE: a population-based, case-control study

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Background: Women with a high pre-pregnancy BMI are at greater risk of postpartum venous thromboembolism (VTE). Whether this is true for BMI at delivery is unknown.

Aims: To evaluate the risk of postpartum VTE associated with pre-pregnancy BMI and with delivery BMI.

Methods: In a population-based, case-control study in Washington State, we identified all females with ICD-9 codes for VTE in the postpartum period (up to 3 months after delivery) between 2003 and 2011. Controls were women without VTE in the postpartum period, matched by birth year to cases. Pre-pregnancy and delivery BMI and covariates were abstracted from birth certificates. Logistic regression models estimated relative risks between categories of BMI, adjusted for maternal and obstetrical characteristics.

Results: Mean (SD) BMI increased from 29.8 (8.1) kg m⁻² pre-pregnancy to 34.7 (8.2) kg m⁻² at delivery (average gain = 13.1 kg) among cases ($n = 291$), and from 26.3 (6.4) kg m⁻² pre-pregnancy to 31.4 (6.2) kg m⁻² at delivery (average gain = 13.6 kg) among controls ($n = 4275$). All pre-pregnancy categories of overweight and obesity were associated with a greater risk of postpartum VTE than the modal BMI category of 18.5–25 kg m⁻²: adjusted OR = 1.5 (95%CI 1.1–2.0) for BMI 25.1–29.9; 2.1 (1.5–3.1) for BMI 30–34.9; 1.8 (1.1–3.0) for BMI 35–39.9; and 4.0 (2.8–6.2) for BMI ≥ 40 . At delivery, only women with a BMI of 35–39.9 or ≥ 40 were at greater risk than women with a BMI in the modal category at delivery of 25–30 kg m⁻²: adjusted OR = 1.7 (1.2–2.5) and 2.7 (1.9–4.0), respectively.

Conclusion: Higher BMIs at pre-pregnancy and delivery are associated with greater risks of postpartum VTE. However, at delivery, only a very high BMI appears to be an independent risk factor. Pre-pregnancy BMI, with a gradually stronger risk for overweight and obese women, may be simpler to use for VTE risk stratification.

Disclosure of Interest: None declared.

OR035

Higher frequency of left-sided deep vein thrombosis in postpartum periodVenemans-Jellema A¹, Elbers L^{2,3}, Middeldorp S³, Gerdes V² and Cannegieter S¹¹Clinical Epidemiology, Leiden University Medical Center, Leiden; ²Internal Medicine, Slotervaart Hospital; ³Vascular Medicine, Academic Medical Center, Amsterdam, Netherlands

Background: The risk of deep vein thrombosis (DVT) is 4- to 5-fold increased during pregnancy and 20-fold increased during the postpartum period compared with non-pregnancy. During pregnancy, the preferred side is the left leg (88%), while a left-sided DVT is only slightly more common (about 55% > 45%) outside pregnancy. It is not known if this left-sided predominance is also present during the postpartum period. This information may indicate whether postpartum DVT results mainly from a thrombus that developed during pregnancy (mechanical cause) or from other causes such as a hypercoagulable state or vascular damage during childbirth.

Aims: To determine the left-right distribution of DVT in women up to 6 weeks postpartum, compared with that in pregnant and non-pregnant women.

Methods: We used data from the MEGA-study, a large case-control study into risk factors for venous thrombosis (consent and ethical approval obtained). We selected female participants younger than 50 years of age, who did not use oral contraceptives and had no cancer. As estimates of relative risks, odds ratios (OR) and 95% confidence intervals were calculated.

Results: 253 DVT patients (58 postpartum, 33 pregnant and 159 non-pregnant) were included. The left-right distribution was 69–31% in the first 6 weeks postpartum compared with 85–15% during pregnancy and 55–45% in non-pregnant women. In week 1–3 postpartum, 71% of the DVTs were left-sided, while this proportion decreased to 60% in week 4–6. Compared with non-pregnancy, the risk of left vs. right sided DVT was 1.8-fold (95%CI 1.0–3.5) higher in women in the postpartum period.

Conclusion: The left-sided predominance present during pregnancy decreased somewhat in the postpartum period, but was still different from that in non-pregnant women. These findings suggest that some DVTs occurring during the postpartum period have developed already before childbirth and have taken some time to cause clinical symptoms.

Disclosure of Interest: : None declared.

Animal models

OR036

Advanced imaging techniques show progressive arthropathy following experimentally induced knee bleeding in a factor VIII^{-/-} rat modelSørensen KR¹, Roepstorff K², Petersen M², Wiinberg B¹, Hansen AK³, Skov S³ and Nielsen LN¹¹Translational Haemophilia Pharmacology; ²Histology & Bioimaging, Novo Nordisk A/S, Maaloev; ³Veterinary Disease Biology, University of Copenhagen, Copenhagen, Denmark

Background: Joint pathology is most commonly assessed by radiography, but ultrasonography (US) is increasingly recognized for its accessibility, safety and ability to show soft tissue changes, the earliest indicators of haemophilic arthropathy (HA). US, however, lacks the ability to visualize the extent of bone deformation. Here advanced radiography such as computed tomography (CT) may play an important part. Little is known about the early phases in the development of HA. We recently developed a Factor VIII^{-/-} (FVIII) rat model and

combining micro-CT (μ CT) with US in this model allows for studies of early joint changes and progression of disease *in vivo*.

Aims: (i) Characterize joint changes in HA using US and μ CT in a new haemophilic FVIII^{-/-} rat and compare to human HA. (ii) Establish the potential of these techniques as tools for assessing progression of HA *in vivo*.

Methods: 20 wild type and 40 FVIII^{-/-} rats received a single joint injury to one knee on day 0 and were euthanized on day 14, or two joint bleeds, on day 0 and 14, and euthanized on day 28. The injured knee of each rat was examined by US for changes in the patella ligament and fat pad, oedema or bone and cartilage degeneration, and *in vivo* μ CT on day 0, 14 and 28. Post mortem, both legs were scanned *ex vivo* by μ CT and examined by histopathology.

Results: FVIII^{-/-} rats developed pathophysiological changes comparable to human HA. US changes were found in all parameters, and in some cases with pathological progression from day 14 to 28. Likewise, μ CT scans revealed dramatic intra- and periarticular changes in the bones.

Conclusion: Joint bleeding in FVIII^{-/-} rats led to joint deterioration that was histopathologically comparable to human HA and evident on both US and μ CT. These new imaging techniques can be used to assess soft tissue and bone deformation in the progression of HA *in vivo* and are promising tools for studying cause and mechanisms driving the pathological changes following haemarthrosis.

Disclosure of Interest: K. Rothaus Sørensen Employee of: Industrial PhD at Novo Nordisk A/S, K. Roepstorff: None declared, M. Petersen: None declared, B. Wiinberg: None declared, A. Hansen: None declared, S. Skov: None declared, L. Nielsen: None declared.

OR037

Development of a mouse carotid artery thrombolysis model for the evaluation of therapeutic approaches for strokeSchoenwaelder SM^{1,2}, Samson AL³, Sturgeon S³, Lee JA^{1,2}, Ju A^{1,2} and Jackson SP^{1,2}¹Charles Perkins Centre, University of Sydney, Camperdown, NSW; ²Heart Research Institute, Newtown, NSW; ³Australian Centre for Blood Diseases, Monash University, Melbourne, Australia

Background: Thrombolytic therapy to achieve reperfusion of occluded blood vessels is the cornerstone therapy for the management of acute ischemic stroke. Despite this, reperfusion is achieved in only 40–50% of patients, with a third of culprit vessels reoccluding, and intracerebral haemorrhage occurring in up to 5% of patients. The development of safe, more efficacious approaches has been hampered by a paucity of preclinical animal models enabling assessment of thrombolysis in large vessels, end-organ damage, bleeding complications and overall neurological deficit.

Aims: To develop an *in vivo* recovery mouse model of *in situ* carotid artery thrombolysis enabling assessment of thrombolytic efficacy, whilst at the same time allowing for the evaluation of cerebral infarct volume and neurological deficit.

Methods: We have established an *in situ* Carotid Artery Thrombolysis (iCAT) model with 90–95% post-operative recovery rate. iCAT was induced by electrolytic injury, that when paired with transient occlusion of the contralateral carotid artery, caused cerebral infarction and commensurate neurological impairment (Bederson score, open field analysis) in mice.

Results: Our studies reveal multi-focal lesions in the brain and reproducible neurological deficit following iCAT, similar to that observed with the Middle Cerebral Artery occlusion (MCAo) model. Histological analysis of carotid arteries confirmed formation of platelet- and fibrin-rich thrombi 24 h post-injury. These thrombi were partially lysed by administration of t-PA or integrilin post-carotid occlusion,

leading to a partial decrease and brain injury and a mild reduction in neurological impairment.

Conclusion: iCAT represents a distinct mouse recovery model that enables assessment of arterial large vessel thrombolysis, brain injury, neurological impairment and bleeding risk. The ability to monitor each of these key variables in a single preclinical animal stroke model should assist with the future development of safer and more effective thrombolytic approaches.

Disclosure of Interest: None declared.

OR038

Real time intravital imaging of thrombus formation in the mouse femoral artery reveals critical differences from events in the microvasculature

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Background: Although clinically-relevant thrombosis commonly occurs in large arteries and veins, current techniques for imaging these events by intravital microscopy have been limited to the microcirculation because they are unable to resolve events in larger vessels possessing thicker and more complex vascular walls.

Aims: Here we report the initial results obtained with a new technique that allows for real time, high-resolution fluorescence imaging in mouse femoral arteries.

Methods: We used confocal microscopy to image hemostatic thrombus formation in surgically-exposed mouse femoral arteries, comparing the results with those obtained previously in studies performed in the cremaster muscle and mesenteric microcirculation.

Results: We observed that thrombi generated in femoral arteries form a core of highly-activated, P-selectin(+) platelets and fibrin, covered by an unstable shell of P-selectin(-) platelets, similar to what we and others have observed in the microvasculature. However, core formation was much slower than in the microcirculation, suggesting a delay in thrombin accumulation. When thrombin was blocked with hirudin, we observed no fibrin accumulation and a 50% decrease in platelet activation, but no measureable effect on total platelet accumulation, unlike the microvasculature where hirudin greatly limits platelet accumulation. Inhibition of ADP signaling caused a large reduction in platelet accumulation, but had no effect on core formation or fibrin deposition.

Conclusion: Our results demonstrate the feasibility of performing real time, high resolution fluorescence imaging in femoral arteries, and indicate that there are critical differences in the hemostatic response in micro- and macrovasculature. Those difference include the kinetics of platelet activation within thrombi and the contribution of thrombin. We propose that this technique will prove informative in understanding events in clinically-relevant vascular diseases, including atherothrombosis.

Disclosure of Interest: None declared.

OR039

Genome editing of factor X in zebrafish reveals unexpected early survival and late onset hemorrhage

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Background: Factor X (F10) deficiency is a rare inherited bleeding disorder with a heterogeneous phenotype and limited therapeutic options. Absence of F10 in mice is embryonic/neonatal lethal, and thus difficult to study. The external development of zebrafish, its ability to generate thousands of offspring at low cost, and optical transparency all make it a powerful tool to study coagulation disorders.

Aims: Generate a model of severe hemorrhage using genome editing in zebrafish.

Methods: Targeted knockout of the zebrafish *f10* locus using TALENs.

Results: Although indistinguishable morphologically from *f10*^{+/+} and *f10*^{+/-} siblings at early stages, *f10*^{-/-} mutants demonstrated a bleeding phenotype with progressive lethality between 1–6 months of age and extensive hemorrhage in multiple tissues, particularly brain. The *f10*^{-/-} mutants did not develop thrombi in response to laser-mediated venous endothelial injury, and this was rescued by transient expression of wild type zebrafish *f10*. Known human F10 disease causing substitutions G222D and C350F were engineered into the orthologous positions in the zebrafish *f10* cDNA, and each failed to rescue the bleeding phenotype. We proceeded to screen sequence variants from 5 newly identified patients with F10 deficiency, and were able to localize the causative mutation in each individual.

Conclusion: We have produced a zebrafish model of human F10 deficiency that exhibits a spontaneous adult lethal bleeding phenotype, yet early embryonic survival is unaffected, in contrast to mice. Further study of this mutant may identify species specific factors enabling this early survival. This platform may also be useful to screen variants from patients with F10 deficiency and other coagulation disorders, as well perform small molecule screens to identify novel therapeutics.

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OR040

Antibody response to human recombinant factor VIII in a new rat model of hemophilia ALövgren KM^{1,2}, Soendergaard H³, Skov S², Weldingh KN⁴ and Wiinberg B¹¹Translational Hemophilia Pharmacology, Novo Nordisk A/S, Maaloev; ²Department of Veterinary Disease Biology, University of Copenhagen, Frederiksberg; ³Diabetes Complications Pharmacology; ⁴Immunogenicity Assessment, Novo Nordisk A/S, Maaloev, Denmark

Background: Neutralizing isoantibodies toward FVIII therapy in hemophilia A (HA), i.e. inhibitors, is an unwanted complication that affects around 30% of severe HA patients. A rat model of severe HA (F8^{-/-}) has recently been developed and the phenotype has previously been described, but an immunological characterization is needed to determine the value of using the model in inhibitor research.

Aims: To characterize the antibody response (binding and neutralizing) towards human coagulation factor VIII (hFVIII) in the HA rat model following a human prophylactic dosing regimen.

Methods: Two identical studies were performed; including a total of 17 homozygous HA rats (0% FVIII activity), 12 heterozygous and 12 wild type littermates. All rats received intravenous injections of recombinant hFVIII (rhFVIII) at 50 IU kg⁻¹ twice weekly for 4 weeks. Blood samples were obtained via the tail vein once weekly for 7 weeks. Binding anti-rhFVIII antibodies were analyzed using a radioimmunoassay detecting binding anti-rhFVIII IgG in rat plasma. Neutralizing anti-rhFVIII antibodies were analyzed using a Bethesda-like assay.

Results: In both studies, binding antibodies developed after 4–6 administrations of rhFVIII (2–3 weeks), with measurable neutralizing antibodies emerging roughly 1 week later, reaching 1–111 BU mL⁻¹ (mean 19 BU mL⁻¹) at end of study. There was no significant difference between the two studies (Mann-Whitney test, $P < 0.05$) or between genotypes in time to response or levels reached for binding and neutralizing antibodies (Kaplan-meier and Friedman test, $P < 0.05$). Repeated intravenous injections and blood samplings via the tail vein were well tolerated.

Conclusion: Following intravenous administration of human FVIII, according to a clinical prophylaxis regimen, a robust and reproducible antibody response is seen in this HA rat model, suggesting that the model is useful for intervention studies with the aim of suppressing, delaying or preventing the inhibitor response.

Disclosure of Interest: K. Lövgren Employee of: University of Copenhagen, H. Soendergaard Employee of: Novo Nordisk A/S, S. Skov Employee of: University of Copenhagen, K. Weldingh Employee of: Novo Nordisk A/S, B. Wiinberg Employee of: Novo Nordisk A/S.

Endothelium and vessel wall

OR041

Autophagy regulates thrombus formation in mice

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Background: Endothelial cells (ECs) can trigger arterial thrombosis through the release of prothrombotic molecules upon vascular injury. However, the mechanism by which ECs cause thrombosis is not entirely understood. Previous studies have shown that loss of autophagy, an intracellular catabolic process, results in decreased secretion of von Willebrand factor in cultured ECs and in mice (Nat Med 2013;19:1281-1287). This finding demonstrates that autophagy may play an important role in mediating the prothrombotic response.

Aims: To further explore the role of autophagy in thrombus formation, we used a Cre-Lox system to generate endothelial cell-specific

deletion of ATG7 (EC-ATG7^{-/-}) in mice. ATG7 is a critical component of autophagy.

Methods: EC-ATG7^{-/-} mice were used to evaluate thrombus formation in a laser injury of the cremaster arteriole. In a separate set, blood was collected to measure platelet count in a hemocytometer and platelet surface expression of P-selectin and β 3-integrin (β 3) by fluorescence-activated cell sorting.

Results: Thrombus formation was examined in 38 and 33 thrombi from wild-type (WT) and EC-ATG7^{-/-}, respectively. The time to maximum intensity in EC-ATG7^{-/-} mice was significantly prolonged compared with WT mice (1.0 ± 0.6 and 2.3 ± 1.2 min, respectively; $P < 0.001$). Thrombi from WT mice were significantly larger than thrombi from EC-ATG7^{-/-} mice, as demonstrated by platelet fluorescence intensity (8.2 ± 2.9 and 1.8 ± 0.7 , respectively; $\times 10^9$; $P < 0.05$). The platelet counts for 11 WT and 11 EC-ATG7^{-/-} mice were not significantly different (768 ± 86 and 715 ± 125 , respectively; $\times 10^6$; $P = 0.3$). Surface expressions of P-selectin and β 3 in WT and EC-ATG7^{-/-} were also similar.

Conclusion: EC-ATG7^{-/-} mice demonstrate decreased thrombosis in the cremaster thrombosis model whereas platelet count and expression of P-selectin and β 3 expression remained normal, suggesting that autophagy contributes to thrombosis in a platelet-independent manner.

Disclosure of Interest: None Declared.

OR042

Platelet activation and platelet-endothelial interactions in the SIV-infected pigtail macaque model of HIV-associated CNS diseaseLyons CE¹, Schneider H², Engle EL³, Najarro KM³, Queen SE³, Morrell CN⁴, Mankowski JL³ and Pate KM³¹Tufts University Cummings School of Veterinary Medicine, North Grafton; ²Colorado State University, Fort Collins;³Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, Baltimore; ⁴University of Rochester, Rochester, USA

Background: Platelet decline is associated with increased risk for the development of HIV-associated neurocognitive deficits. Interaction of activated platelets with brain microvascular endothelium may contribute to platelet decline.

Aims: To determine whether activated platelet-endothelial interactions are associated with CNS disease in the SIV-infected pigtailed macaque model of HIV.

Methods: Platelet activation was monitored throughout infection for SIV-infected macaques and uninfected controls. Brains were evaluated to determine CNS disease status and for immunohistochemistry for platelet-endothelial binding and perivascular macrophage cuffs. Confluent monolayers of brain microvascular endothelial cells (BMECs) were exposed to washed platelets or media in transwells and permeability quantified.

Results: Platelets harvested from infected macaques that went on to develop CNS disease during terminal infection demonstrated less activation than macaques without CNS disease during acute ($P = 0.04$) and asymptomatic ($P < 0.0001$) infection. Brains from SIV-infected macaques were more likely than brains from uninfected controls to have platelets bound to vascular endothelium during acute (RR 4.0, $P = 0.03$) and terminal (RR 3.6, $P = 0.04$) infection. 6 of the 10 SIV+ macaques had CNS disease during terminal infection and resident Mac387+ (RR 3.4, $P = 0.0001$) or CD163+ macrophages (RR 1.44, $P = 0.0005$) but not non-resident CD68+ macrophages (RR 1.2, $P = 0.2$) were observed in these animals with increased likelihood around platelet-lined vessels. SIV-infected macrophages were similarly observed with increased likelihood around platelet-lined vessels (RR 1.5, $P = 0.007$). Permeability of BMECs decreased two-fold following

incubation with platelets from SIV infected macaques compared with uninfected macaques ($P = 0.01$).

Conclusion: Activated platelet-endothelial interactions may represent a protective mechanism against development of macrophage infiltrates in CNS disease that is removed in the context of HIV-associated thrombocytopenia.

Disclosure of Interest: None declared.

OR043

Vascular endothelium dysregulation following staphylococcus aureus infection: new insights for sepsis

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Background: Sepsis is one of the major causes of mortality in critically ill patients and is commonly caused by *Staphylococcus aureus*. The endothelium is a major target of sepsis-induced events and vascular endothelial damage accounts for much of the pathology of septic shock. As a first line of defence, endothelial cells recognise invading bacteria through specific interactions however the nature of these interactions is not well defined.

Aims: The aim of this study was to develop a clinically relevant model of infection to study the interactions between human vascular endothelial cells and *S. aureus*.

Methods: In order to mimic physiological conditions experienced in sepsis human aortic endothelial cells (HAECs) were sheared at 10 dynes for 24 h followed by addition of TNF α and human blood plasma. Fluorescently labelled bacterial adherence was measured over a 1 hr time period and cellular response to infection was measured over a 24 hr time period.

Results: *S. aureus* bound significantly more to HAEC's when they were subjected to physiological shear and were treated with TNF α and human plasma (439% more binding than in the absence of shear, TNF α or plasma, $P < 0.05$, $n = 3$). Clumping factor A (ClfA) is the most abundant protein expressed on *S. aureus* and deletion of this protein significantly reduced adhesion to the HAEC's (70% inhibition compared to parent strain, $P < 0.001$, $n = 3$). ClfA binds fibrinogen to bridge to host cells and removing fibrinogen from plasma resulted in a significant reduction in binding (53% $P < 0.05$, $n = 3$). *S. aureus* infection dysregulates HAEC's, HAEC proliferation was significantly reduced (72% inhibition, $P < 0.001$, $n = 3$); apoptosis and permeability of HAEC's was significantly increased (33% increase, $P < 0.01$, $n = 3$, 67% increase, $P < 0.01$, $n = 3$, respectively), all consistent clinical signs of sepsis. Using a strain defective in ClfA reversed all of these effects.

Conclusion: In conclusion, *S. aureus* ClfA is responsible for triggering a number of events that result in endothelial dysfunction in sepsis.

Disclosure of Interest: None declared.

OR044

Endothelial BAMBI (BMP and activin membrane bound inhibitor) is important for fibrin generation and thrombus stability

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Background: BAMBI is a transmembrane protein related to the transforming growth factor- β superfamily, and is highly expressed in platelets and endothelial cells (EC). Using *Bambi*-deficient mice, we recently showed that BAMBI plays a role in haemostasis and also in thrombus stability. Using chimeric mice, we also demonstrated that it is most likely BAMBI present in the endothelium and not in platelets that influences the haemostatic response and thrombus stability.

Aims: This study aims to delineate the mechanisms by which BAMBI exerts its function on the endothelium and how these influence thrombus formation and stability.

Methods: Mouse plasma prostacyclin and nitric oxide (NO) levels were determined using *in vitro* assays. Mouse lung endothelial cells (MLEC) were isolated from *Bambi*^{+/+} and *Bambi*^{-/-} mice and expression of adhesion molecules was assessed by flow cytometry. Mice were subjected to the laser-induced thrombosis model of the cremaster arterioles.

Results: While no difference in levels of prostacyclin could be observed, plasma NO levels were significantly increased (~50%) in *Bambi*^{-/-} mice compared to wild-type littermates. Results from separate MLEC isolations showed that *Bambi*^{-/-} MLEC expressed lower levels of EC adhesion molecules PECAM1 and ICAM2 compared to *Bambi*^{+/+} MLEC. Finally, after laser injury of the endothelium, *Bambi*^{-/-} mice exhibited thrombus instability accompanied with a defect in fibrin(ogen) accumulation compared to *Bambi*^{+/+} littermates.

Conclusion: We provide first evidence on how endothelial BAMBI may influence thrombus stability and hemostasis by modulating NO release, and the expression levels of EC adhesion molecules. We also demonstrate that *Bambi*-deficient mice exhibit impaired fibrin deposition during thrombus formation potentially suggesting a defect in thrombin formation. We are currently investigating the level of endothelial and platelet activation after laser injury of the endothelium in *Bambi*-deficient mice as well as the effect of hirudin on thrombus formation and stability.

Disclosure of Interest: None declared.

OR045

Validating A 3D tissue-engineered human blood vessel model for use as a novel model system for the assessment of thrombus formation

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Background: Thrombus formation is a complex process involving multiple factors that is commonly studied either *ex vivo* or *in vivo* animal models. A 3D tissue engineered blood vessel (TEBV) could provide a superior system to analyse blood clotting in a viable human blood vessel.

Aims: Here we have designed, constructed and validated a TEBV to assess whether it could be used as a new model to study thrombus formation under physiologically relevant conditions.

Methods: TEBVs were constructed from an intimal layer consisting of Human Umbilical Vein Endothelial Cells (HUVECs) grown upon aligned nanofibers, and a medial layer composing of human coronary artery smooth muscle cells (HCASMCs) grown in a collagen hydrogel.

The confluency of the HUVECs layer was assessed by tracking permeation of 150K fluorescein dextran. The physiological state of the HUVECs was examined using immunohistochemistry of ICAM-1 expression. A new protocol was also developed to measure platelet activation when exposed to the TEBV using fluorescence spectrophotometer. This allows *in situ* real-time monitoring of the state of platelets manifested as of changes in their cytosolic calcium concentration ($[Ca^{2+}]_{cyt}$) where the platelets have been dually-labelled with both Fura-2/AM and DiOC₆.

Results: The HUVECs layer in the cultured TEBV model possessed high confluency, anti-thrombogenicity and phenotype similar to that found *in vivo*. The TEBV resists dextran permeation through the HUVECs monolayer with minimum expression of ICAM-1. Together, the TEBV construct inhibit thrombus formation on the HUVECs layer. As a result, the exposure of platelets to the multi-layered TEBV construct elicited a significant reduction in the thrombin-evoked rise in $[Ca^{2+}]_{cyt}$ ($36.7 \pm 11.7\%$ of control; $n = 6$; $P < 0.05$), when compared to samples not exposed to the construct.

Conclusion: We have successfully created a TEBV that is able to replicate the functional properties of the native vessel and which may be useful as a novel model to study human thrombus formation.

Disclosure of Interest: None declared.

Tissue factor – factor VII – I

OR046

Cross-talk between tissue factor and EPHA2 in cancer: potentiation of ligand-dependent EPHA2-signaling *in vitro* and co-expression in human colorectal cancer specimens

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Background: Tissue Factor (TF) forms a proteolytically active complex with coagulation factor VIIa (FVIIa) and functions as the trigger of blood coagulation or alternatively activates cell signaling. We recently described that EphA2 of the Eph tyrosine kinase receptor family is cleaved directly by the TF/FVIIa complex independently of PAR2 and downstream coagulation proteases.

Aims: The aim of the present study was to further characterize the cross-talk between TF/FVIIa and EphA2 using *in vitro* model systems and a cohort of colorectal cancer specimens.

Methods: Cleavage and serine phosphorylation of EphA2 was studied by Western blot. Subcellular localization of TF and EphA2 was investigated by a proximity ligation assay and confocal microscopy. Phalloidin staining of the actin cytoskeleton was used to study cell rounding and retraction fiber formation in response to the EphA2 ligand ephrin-A1. Immunohistochemistry was used to examine expression of TF and EphA2 in human colorectal cancer specimens.

Results: FVIIa-stimulation resulted in serine-897 phosphorylation and direct cleavage of EphA2 in MDA-MB-231 cells. TF and EphA2 co-localized constitutively in MDA-MB-231 cells at cell-cell contacts together with polymerized f-actin. Transient overexpression of TF in U251 glioblastoma cells lead to co-localization with EphA2 at the leading edge and FVIIa-dependent cleavage of EphA2. FVIIa stimulation potentiated ephrin-A1-induced cell rounding and retraction fiber formation in a RhoA/ROCK-dependent manner. TF and EphA2 expression correlated in colorectal cancer (Spearman Rho 0.48, $P < 0.001$), where 90% of TF positive cases also expressed EphA2.

Conclusion: These results demonstrate that TF-EphA2 cross-talk potentiates ligand-dependent EphA2 signaling in human cancers, and provide initial evidence that it is possible for this interaction to occur

in vivo. Our results provide further evidence for a novel direct signaling mechanism for the TF/FVIIa complex through cross-talk with the EphA2 receptor

Disclosure of Interest: None declared.

OR047

Antibody-based targeting of alternatively spliced tissue factor impedes the growth and aggressiveness of pancreatic ductal adenocarcinoma

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Background: Alternatively spliced Tissue Factor (asTF) is upregulated in pancreatic ductal adenocarcinoma (PDAC). asTF is minimally coagulant and signals non-proteolytically via $\beta 1/\beta 3$ integrins; asTF's overexpression fuels PDAC growth, metastatic spread, monocyte recruitment, and elevated pro-coagulant potential of PDAC cells.

Aims: To determine if monoclonal antibody-based targeting of asTF slows PDAC progression and/or asTF release into circulation in an orthotopic setting.

Methods: Nude and, in some experiments, SCID and SCID/lowTF mice were transplanted with human PDAC cells Pt45P1 and tumor growth monitored *in vivo* via SapC-DOPS imaging. Plasma asTF was assessed using a prototype ELISA. 100 μ g of anti-asTF monoclonal antibody Rb1 was co-implanted with Pt45P1 cells and vessel density (anti-CD31); monocyte/macrophage infiltration (anti-F4/80 & anti-CD206); and collagen-rich tumor stroma (Masson-Trichrome) were assessed. One-way ANOVA was used to assess significance, set at < 0.05 .

Results: PDAC cells co-implanted with Rb1 grew significantly smaller tumors that were i) ~3 fold less vascularized, ii) had ~3.5 fold fewer stromal macrophages compared to the cells co-implanted with vehicle (PBS) and/or isotype control IgG, and iii) had ~30% reduction in collagen-rich stroma content. Mice in the Pt45P1/Rb1 cohort had a ~2 fold decrease in the levels of circulating asTF (0.27 ng mL⁻¹ vs 0.54 ng mL⁻¹ in the IgG cohort). Pt45P1 cells co-implanted with Rb1 in SCID mice also grew tumors significantly smaller compared to those in the IgG control group; the effects of Rb1 on primary tumor growth in SCID/lowTF mice were similar to those in SCID mice.

Conclusion: In the presence of Rb1 in nude as well as SCID mice, Pt45P1 cells grow significantly smaller tumors with less stroma and fewer monocytes/blood vessels that release less asTF protein in the systemic circulation. Antibody-based targeting of asTF may comprise a novel strategy to fight PDAC with a low risk of bleeding.

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OR048

Tissue factor inhibition attenuates neuronal damage and behavioral deficit in a mouse model of ischemic stroke

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Background: Astrocytes are the main source tissue factor (TF) in the brain. Using mouse models of hemorrhagic and ischemic stroke, we previously reported that astrocyte TF is essential for brain hemostasis but also contributes to brain injury via a thrombo-inflammatory mechanism. Using mice with different levels of astrocyte TF expression, we showed that astrocyte TF activity around 5% of the wild type level was sufficient to limit bleeding in hemorrhagic stroke and at the same time was low enough to reduce neuronal injury without increasing hemorrhagic transformation.

Aims: Determine the effects of pharmacologic inhibition of TF in a mouse model of ischemic stroke.

Methods: C56Bl/6J mice were subjected to middle cerebral artery occlusion (MCAO) for 1 h followed by 24 h of reperfusion. Inhibitory rat anti-mouse TF antibody (1H1) and control IgG were given as a bolus intraperitoneal injection immediately after ischemia.

Results: 1H1 dose-dependently reduced brain infarct size in female mice subjected to MCAO ($36.6 \pm 1.4\%$ of total brain volume in IgG group [$n = 19$] vs $28.3 \pm 1.7\%$ in 25 mg kg^{-1} 1H1 group [$n = 9$, $P < 0.01$] and $18.5 \pm 2.3\%$ in 75 mg kg^{-1} 1H1 group [$n = 8$, $P < 0.001$]; mean $\pm$ SEM). Importantly, blocking TF did not exacerbate hemorrhagic transformation during the reperfusion phase. Compared to the IgG treated group, 1H1 significantly reduced levels of inflammatory markers (IL-6, MCP-1 and KC levels—analyzed by ELISA) and microvascular thrombosis (analyzed by fibrin staining) in the injured brain as well as attenuated behavioral deficit after ischemic stroke. Male mice subjected to the same MCAO conditions experienced high mortality in the IgG treated group (4 out of 6 mice died with 24 h) which was eliminated by 1H1 (25 mg kg^{-1}) treatment (0 out of 5, Log-rank test $P = 0.031$).

Conclusion: Our data demonstrate that during the reperfusion phase following ischemic stroke, TF contributes to neuronal damage via both pro-inflammatory and pro-thrombotic mechanisms. Blocking TF did not increase hemorrhagic transformation.

Disclosure of Interest: S. Wang: None Declared, B. Reeves: None Declared, D. Kirchhofer Employee of: Genentech Inc, R. Pawlinski: None declared.

OR049

Low factor VII levels fail to reduce vascular graft thrombus propagation in a baboon model

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Background: The tissue factor/factor VII (TF/FVII) complex-dependent extrinsic pathway of thrombin generation has been considered essential to the pathogenesis of thrombosis. Inhibition of the extrinsic pathway was found to be antithrombotic in several animal models over the past 3 decades, however, FVII- or TF-targeting drugs have not been commercialized.

Aims: This study was designed to determine whether low circulating FVII levels are safe and antithrombotic in a primate model of acute vascular graft thrombosis.

Methods: Hemostasis impairment was assessed as increase in bleeding time and volume. Reduction of FVII plasma levels was achieved by parenteral generation 2.5 FVII antisense oligonucleotide (FVII ASO) treatment of baboons. Thrombogenesis was quantified by measuring platelet and fibrin deposition in collagen- and tissue factor-coated vascular grafts (4 mm ID, 20 mm long) that were deployed into arteriovenous shunts and exposed to 100 mL min^{-1} pulsatile flow for 60 min. Reduction of FVII levels to $< 1\%$ was not pursued due to a risk of severe bleeding. Enoxaparin (1 mg kg^{-1}) was used as positive control.

Results: Enoxaparin anticoagulation was antithrombotic. FVII ASO treatment increased prothrombin time to > 2 -fold and reduced FVII levels to $\approx 5\%$ of baseline within 2 weeks. Platelet deposition was not inhibited in collagen-coated grafts, while it was only marginally delayed and reduced in TF-coated grafts by $\approx 20\%$. Fibrin deposition was not inhibited. Bleeding time and volume were marginally increased.

Conclusion: We conclude that if FVII contributed to thrombus propagation indeed, very low FVII levels ($> 0\%$ to $< 5\%$) could be sufficient to sustain thrombogenesis, regardless whether the initiating trigger is collagen or TF, in primates. Our data do not support the hypothesis that the extrinsic pathway substantially contributes to thrombus growth in large vessels.

Disclosure of Interest: M. Wallisch Employee of: Aronora, Inc., J. Crosby Employee of: ISIS Pharmaceuticals, Inc., J. Greisel: None Declared, S. Murray Employee of: ISIS Pharmaceuticals, Inc., E. Tucker Employee of: Aronora, Inc., O. McCarty: None Declared, M. Hinds: None Declared, B. Monia Employee of: ISIS Pharmaceuticals, Inc., A. Gruber Employee of: Aronora, Inc.

OR050

Genetic determinants of activated factor VII-antithrombin complex plasma concentration include tissue factor, factor VII and endothelial protein C receptor gene variants

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Background: Plasma concentration of activated factor VII (FVIIa)-antithrombin (AT) complex has been proposed to be a thrombophilic biomarker, potentially reflecting tissue factor (TF) exposure and TF-FVIIa interaction. Endothelial protein C receptor (EPCR) has been shown to bind FVIIa, but the physiological significance of this interaction in hemostasis is unclear.

Aims: To reveal determinants of the FVIIa-AT plasma concentration exploiting functional gene polymorphisms, known to modulate F7, TF and EPCR levels.

Methods: We evaluated in 605 subjects (75.0% males, mean age 61.0 ± 10.9 years, 80.0% with coronary artery disease) FVIIa-AT plasma levels (ELISA assay) and genotypes for TF (-603 A>G and +5466 A>G), F7 (-402 G>A, -323 A1/A2 and R353Q), and EPCR (S219G).

Results: F7 -323A2 and 353Q allele carriers and TF -603GG-homozygotes had lower FVIIa-AT complex levels. Differently, EPCR 219G minor allele carriers had higher levels. Including the polymorphisms in a linear regression model, F7 -323A1/A2 ($\beta = -0.226$; $P < 0.001$), TF -603 A>G ($\beta = -0.126$; $P = 0.001$) and EPCR S219G ($\beta = 0.080$; $P = 0.044$) polymorphisms remained significant predictors of FVIIa-AT concentration. They explained an overall level variability (6.7%) similar to that (7.1%) of other major determinants, i.e. renal function ($\beta = -0.194$; $P < 0.001$) and plasma lipids (HDL-cholesterol: $\beta = 0.195$; $P < 0.001$; triglyceride: $\beta = 0.117$; $P = 0.011$). The genetic

contribution was virtually unchanged after adjustment for sex, age, CAD diagnosis, as well as renal function and plasma lipids.

Conclusion: Our results show the interplay of several genetic components as independent determinants of FVIIa-AT plasma concentration, and indicate for the first time the contribution of EPCR gene variants. Since the investigated polymorphisms modulate proteins directly involved in FVIIa-AT complex formation and potentially influence their interaction, our study may add novel elements to understand the regulation “*in vivo*” of the FVII-TF pathway.

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Heparin-induced thrombocytopenia

OR051

Dynamic mechanical thromboprophylaxis is a major heparin-independent risk factor for the formation of anti-platelet factor 4/heparin antibodies in patients undergoing total knee or HIP arthroplasty

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Background: Spontaneous or fondaparinux-associated heparin-induced thrombocytopenia (HIT) is caused by platelet-activating antibodies (Abs) against platelet factor 4 (PF4)/heparin (H) complexes without H exposure, mostly in orthopedic surgery patients (pts). This implies that surgery itself could trigger this immune response, although the mechanism is unclear.

Aims: We aim to investigate how surgery could trigger an immune response against PF4/H complexes.

Methods: In a multicenter, prospective study of 2069 pts undergoing total hip or knee arthroplasty (TKA or THA), approximately half were treated with unfractionated H (UFH), low-molecular-weight H (LMWH), or fondaparinux. The other half received only mechanical prophylaxis [dynamic: intermittent plantar compression or intermittent pneumatic compression device, static: graduated compression stocking (GCS), or both]. We measured anti-PF4/H IgG/A/M before and 10 days after surgery using an immunoassay.

Results: Multivariate analysis revealed that the dynamic mechanical prophylaxis (DMP), in addition to female sex and TKA (vs. THA), was an independent risk factor for the seroconversion (odds ratio 2, 95% CI 1.3–3, $P = 0.001$), which was confirmed with propensity score matching. For TKA, the seroconversion rate in pts treated with DMT and no anticoagulant (15.3%) and pts treated with UFH (14.8%) or

LMWH (13.7%) without DMP were similar, and it was higher than that in pts treated with fondaparinux without DMP (5.7%) or only GCS (6.4%). The proportion of pts with > 1.4 optical density was higher among those treated with any anticoagulant and DMP than among those not treated with DMP, suggesting that DMP may also act as a co-stimulator in the immune response.

Conclusion: We propose a novel hypothesis that DMP is involved in the immune response against PF4/H independent of H, probably due to stimulation of PF4 release and production of polyanions such as glycosaminoglycans and nucleic acids with tissue damage, which is presumably associated with the risk of developing HIT.

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OR052

Is the incidence trend of heparin-induced thrombocytopenia decreased by the increased use of low-molecular-weight-heparin?

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Background: The increasing trend of using low-molecular-weight-heparin (LMWH) vs. unfractionated heparin (UFH) in hospitalized adult patients is raising concerns about the incidence of heparin-induced thrombocytopenia (HIT)

Aims: To assess the effect of prescribing UFH and LMWH on the annual incidence of HIT, with additional analysis of the annual lab requests for HIT antibodies and confirmed positive HIT tests at a single teaching center

Methods: A retrospective study analyzed the requests for heparin-induced antibodies by enzyme-linked immunosorbent assay (ELISA) among adult hospitalized patients during the period from January 2011 to December 2013. These patients received either UFH or LMWH for prevention or therapeutic indications. Those with positive immune-mediated HIT were identified and considered as case patients

Results: The main clinical demographic characteristics of the 116 patients who developed HIT were determined. In patients receiving UFH and those receiving LMWH, the annual incidence rate of HIT per one thousand patients was 4.42 and 0.46 in 2011 ($P < 0.0001$); 4.19 and 0.47 in 2012 ($P < 0.0001$); 3.48 and 0.50 in 2013 ($P < 0.0001$), respectively, with an over 3-year incidence of 4.09 and 0.48 ($P < 0.0001$) respectively. The patients who received UFH were 8.5 times more likely to develop HIT than those who received LMWH. A decrease in the total annual incidence rate of HIT, UFH and LMWH, was observed: 3.24 in 2011, 2.62 in 2012 and 1.72 in 2013. The difference between the incidence in 2011 and 2013 was statistically significant (difference = 1.53, 95%CI 0.36 to 2.71, $P = 0.006$). The annual number of patients receiving LMWH inversely correlated with annual number of HIT assays performed, while the annual number of patients receiving UFH correlated very closely with the annual numbers of HIT assays performed

Conclusion: In this three-year study period, we identified a decreasing incidence rate of HIT in hospitalized adults patients which may be attributed to the increasing use of LMWH over UFH

Disclosure of Interest: None Declared.

OR053

The impact of optical density cut-off on sensitivity and specificity for the diagnosis of heparin induced thrombocytopenia: a systematic review and meta-analysis

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Background: The current diagnostic approach to heparin induced thrombocytopenia (HIT) relies on screening suspected patients with immunological tests for the presence of anti-platelet factor 4 antibodies. These assays are limited by poor specificity, leaving many patients with clinically suspected HIT to await results of less readily available but more specific functional platelet activating assays. It has been suggested that increasing the cutoff for interpreting immunological assays can increase the specificity without significantly compromising false negative rate.

Aims: We performed a prospectively registered systematic review and meta-analysis to estimate the sensitivity and specificity of immunological at varying optical density (OD) cutoff points.

Methods: We searched MEDLINE, EMBASE and CENTRAL databases as well as abstracts from major scientific conferences and hand search of references. Studies were included if they contained consecutive patients with clinically suspected HIT and underwent both immunological and functional HIT testing. Quality of included studies was assessed by the QUADAS-2 criteria.

Results: A total of 19 studies met our inclusion criteria with a combined patient population of 13,485 patients. The effect of varying the OD cut-off was such that as the OD threshold increases from 0.4 to ≥ 2.0 the sensitivity of the test decreases from 0.93 (95% CI 0.85–0.98) to 0.56 (95% CI 0.21–0.87) and the specificity increases from 0.78 (95% CI 0.72–0.84) to 0.98 (95% CI 0.96–0.99). The best compromise between sensitivity and specificity appears to be with an OD cutoff threshold of between 0.6–0.99, which results in a sensitivity of 0.92 (95% CI 0.90–0.95), specificity of 0.88 (95% CI 0.84–0.92). Significant heterogeneity between studies limits the interpretation of these pooled results.

Conclusion: Although promising, future prospective diagnostic studies would be required before firm recommendations on changing the diagnostic approach to HIT can be made.

Disclosure of Interest: None declared.

OR054

HIT or PIT- clinical relevance of the zymutest HIA IGG (hyphen biomed) in a routine laboratory setting

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Background: A second pathomechanism due to antibodies against protamine-heparin-complex (PIT) resulting in the clinical picture of heparin-induced thrombocytopenia (HIT) was discovered and clinical data have been published (Bakchoul T. 2013). Platelet activation is similar via Fc gamma IIa receptor. The presence of platelet-activating anti-protamine-heparin antibodies prior cardiac surgery is associated

with early postoperative thrombocytopenia and an increased risk of arterial thrombosis.

Aims: To investigate the clinical relevance of the Zymutest HIA IgG (HYPHEN BioMed) in our cohort of patients, to determine prevalence of anti-protamine/heparin antibodies.

Methods: Samples sent to our laboratory (4008 in 21 months) to rule out HIT were tested the same day with a functional assay (mod. aggre-gometry) and Zymutest HIA IgG (Hyphen Biomed; pos result OD > 0.5). Heparin-neutralization procedure (HNP) was performed in positive samples to rule out unspecific antibody (ab)-binding. HNP was performed in patients with high likelihood of previously exposure to protamine sulfate ($n = 96$). When the HNP revealed specific ab binding, we further differentiated if anti-protamine sulphate-heparin ab were present (assay without platelet lysate).

Results: 91.6% (3672 samples) were tested as HIT negative, 2.9% ($n = 118$) weak positive (OD > 0.5) and 3.0% ($n = 122$) were positive (OD > 0.8). Out of 96 HNP-procedures 70 remained positive (specific HIT ab). In 9 samples we could detect ab against protamine-heparin-complex, 17 revealed unspecific binding.

Conclusion: We found ab against protamine-heparin-complex in 9/201 (4.5%) positive samples. Even in patients pre-exposed to protamine, PIT frequency is low in our cohort of patients. With the assumed increase in diabetics, cardiovascular disease and cardiovascular surgery in the population this pathomechanism may become more important.

Disclosure of Interest: None declared.

OR055

Evaluation of flow cytometric assay in the diagnosis of HIT

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Background: Ready access to a rapid and reliable functional assay, not requiring the use of radioactive reagents, is one of the major desires of clinicians faced with a suspicion of heparin-induced thrombocytopenia (HIT).

Aims: Our objective was to evaluate the performance of a functional flow cytometric assay (FCA), compared to that of a serotonin release assay (SRA), with regard to HIT diagnosis.

Methods: This was a multicenter study “HIT score” (NCT00748839) in which the diagnosis of HIT was established by two independent experts (three in the case of discordance) on the basis of clinical and laboratory data. Plasma from 297 patients, randomly selected from the total study population, was analyzed. Of these patients, 139 had a positive HIT diagnosis and 158 were negative for HIT. SRA and FCA were performed centrally. In accordance with published FCA methodology (Tomer, 1997), 10 μ l of control fresh platelet-rich plasma were incubated with 10 μ l of patient plasma in the presence of heparin (0.3 or 100 IU mL⁻¹) or saline. Each sample was incubated with a mixture of a platelet marker (PE-conjugated anti-CD41) and an activation marker (FITC-CD62P mAb). Platelet suspensions were analyzed using a BD Accuri C6 flow cytometer. Moreover, in order to evaluate lab-to-lab variability, samples from 10 patients were sent to five laboratories for analysis according to the FCA protocol.

Results: The sensitivity and specificity of the FCA were respectively 88% (95% CI: 84–91) and 78% (95% CI: 73–83), the sensitivity and specificity of the SRA being respectively 79% (95% CI: 74–84) and 90% (95% CI: 86–93). The FCA was therefore more sensitive than the SRA. The specificity of both assays was greatly improved by coupling to IgG ELISA.

Conclusion: The FCA gave results similar to those of the SRA with respect to HIT diagnosis. Taking into account its potential advantages, FCA seems to represent a promising method for routine practice.

Disclosure of Interest: None declared.

Mechanical circulatory support and devices

OR056

Acquired von willebrand syndrome is a common and progressive condition among pediatric ECMO patients

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Background: Hemorrhagic complications on ECMO are overall more common in pediatrics than their adult counterparts. Acquired von Willebrand syndrome (aVWS) can increase bleeding risk in ECMO patients. However, the prevalence and severity of aVWS among pediatric patients on ECMO has not been well described.

Aims: The aim of this study is to determine the prevalent and characteristics of aVWS in pediatric ECMO patients.

Methods: Thirty three pediatric ECMO patients (age 1 day to 17 year old) were included in this study. Plasma samples were collected on ECMO day 1 and every 5 $\pm$ 3 day until patients were off ECMO. Studies performed included von Willebrand factor (VWF) antigen (VWF:Ag), VWF ristocetin co-factor activity (VWF:RCO), VWF collagen binding assay (VWF:CBA), VWF propeptide (VWF:PP), and ADAMTS13. In addition, VWF CBA to VWF:Ag ratio (CBA/Ag), VWF:RCO to VWF:Ag ratio (RCO/Ag) and VWF:PP to VWF:Ag ratio (PP/Ag) were calculated.

Results: VWF:Ag and VWF:RCO remained > 30% for all patients which indicates quantitatively adequate VWF. However, mean RCO/Ag was 0.5 (normal > 0.7) on day 1 and decreased significantly ($P < 0.05$) to 0.3 on day 12. By day 20 all patients' RCO/Ag were < 0.7. Similarly CBA/Ag was decreased at 0.5 (normal > 0.7) and remained < 0.5. The decreased RCO/Ag and CBA/Ag were indicative of qualitative aVWS secondary to loss of high molecular weight VWF secondary

to high shear in ECMO circuit. Mean PP/Ag and ADAMTS13 levels remained within the normal ranges.

Conclusion: This study demonstrated that progressive aVWS was a common finding among pediatric ECMO patients. The lack of increased PP/Ag could be suggestive of decreased VWF production response to ongoing destruction. Overall, the data suggest that the risk of bleeding may increase during ECMO and supplementation of VWF may be beneficial.

Disclosure of Interest: None declared.

OR057

Comparison of the effect of dabigatran and warfarin on mechanical heart valve-induced thrombin generation

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Background: Patients with mechanical heart valves (MHVs) require lifelong warfarin therapy to prevent thromboembolism. Dabigatran is at least as effective as warfarin for stroke prevention in atrial fibrillation. The RE-ALIGN trial comparing dabigatran with warfarin in patients with MHVs was stopped early because of more strokes and bleeding with dabigatran.

Aims: To determine why dabigatran was less effective than warfarin, we compared their effects on thrombin generation (TG) induced by MHVs.

Methods: TG was quantified in the absence or presence of valve leaflets, or sewing ring segments (SRS). Studies were done in recalcified plasma containing varying concentrations of dabigatran, or from patients on warfarin with varying international normalized ratio (INR) values.

Results: Mean endogenous thrombin potential (ETP) increased from a background of 4401 ± 192 nM·min to 5170 ± 90 , 7951 ± 204 , and 6675 ± 463 in the presence of leaflets, Dacron SRS, and Teflon SRS, respectively. In the presence of leaflets or SRS, ETP in factor (F) VII-depleted and control plasma were similar. In contrast, ETP was reduced to background levels in FXII-depleted plasma and was even lower in FXI-depleted plasma. Dabigatran had little effect on ETP at concentrations below 400 ng mL⁻¹, while warfarin suppressed ETP when INR values exceeded 1.5. Therefore, warfarin attenuates TG induced by MHVs more effectively than dabigatran and dabigatran concentrations of 260 and 480 ng mL⁻¹ would be required to suppress ETP to the same extent as warfarin at INR values of 2 and 3.5, respectively.

Conclusion: MHVs induce TG via the intrinsic pathway and generate thrombin in concentrations that overwhelm clinically relevant concentrations of dabigatran. In contrast, by lowering the levels of FIX, FX and prothrombin, warfarin is more effective than dabigatran at suppressing TG induced by MHVs. Our data suggest that strategies that target FXII or FXI would suppress the root cause of thrombosis in such patients.

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OR058

Assessment of platelet function and hemostasis in neonates undergoing cardiopulmonary bypass

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Background: Excessive bleeding is a significant problem in pediatric patients undergoing surgery with cardiopulmonary bypass (CPB). Thrombocytopenia and platelet function defects contribute to bleeding with CPB in adults and older pediatric patients. Comparable data for neonatal patients is lacking because of the large blood volume requirements of conventional platelet function tests.

Aims: To define changes in platelet count and function in neonates during CPB.

Methods: Neonatal (< 30 day old) patients undergoing cardiac surgery at Children's Hospital of Wisconsin were enrolled. Blood was drawn before surgery (baseline), while on CPB, upon removal from CPB, and upon admission to the cardiac intensive care unit (CICU). Blood product transfusion was begun prior to removal from CPB, but after the second blood draw. Platelet counts and fibrinogen levels were assessed in the clinical diagnostic laboratory. Platelet receptor expression levels and agonist-induced platelet activation were measured by flow cytometry. Rotational thromboelastometry (ROTEM) was performed at the point of care.

Results: Platelet counts, fibrinogen levels, and agonist-induced platelet activation decreased significantly on CPB relative to baseline, coincident with significant decreases in ROTEM EXTEM-MCF (maximum clot firmness) and FIBTEM-MCF. Platelet counts, fibrinogen levels and platelet responses to some agonists rose upon removal from CPB and returned to baseline by the time of admission to the CICU, as did ROTEM EXTEM-MCF. FIBTEM-MCF rose following removal from CPB and at the time of admission to CICU but remained significantly lower than baseline values.

Conclusion: Thrombocytopenia, decreases in fibrinogen, and platelet function defects are associated with CPB in neonatal cardiac surgery patients and are corrected by transfusion following removal from bypass. These changes are reflected by ROTEM. ROTEM and flow cytometry are useful small volume assays of hemostasis and platelet function in neonatal cardiac surgery patients.

Disclosure of Interest: None declared.

OR059

Use of fibrinolytic therapy in treatment of ventricular assist device thrombosis

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Background: A major complication of left ventricular assist device (LVAD) placement includes pump thrombosis, resulting in device failure, stroke, need for emergent LVAD exchange or transplant, and death. Fibrinolytic therapy is the only class of drugs to actively break down thrombus. Pump exchange is preferred to fibrinolytic therapy, but is not always feasible. In May 2012 our hospital standardized an approach to use of t-PA for LVAD thrombosis.

Aims: To describe our cases of HeartMate II® thrombosis treated with t-PA.

Methods: Medical record review of patients with LVAD thrombosis who received endoventricular t-PA. Data collection included patient demographics, medical history, medications, laboratory values, LVAD and hemodynamic measurements, and dates on any adverse outcomes (bleeding, stroke, transplant, pump exchange, or death). Statistics are descriptive and presented as mean with standard deviation.

Results: We identified 6 episodes of LVAD thrombosis treated with t-PA into the left ventricle at a rate of 1 mg min⁻¹ for 30 min. All episodes involved Caucasian males with a mean age of 52.8 ± 5.0 yrs and a BMI of 35.3 ± 4.4. All presented on aspirin and warfarin, with 4/6 presenting with an INR < 2.0. 50% were on dipyridamole. Time from LVAD placement to thrombosis ranged from 52–352 days (mean 161 days). All episodes had major hemolysis with a mean LDH of 1745 ± 443 U L⁻¹. 4/6 episodes (66%) had a successful outcome from t-PA therapy with no adverse outcomes for 6 months post therapy. In the successful cases LDH returned to 762 ± 239 U L⁻¹ within 5 days, pulsatile index improved from 4.7 ± 1.3 to 3.4 ± 1.2, flow improved from 6.4 ± 1.1 to 7.8 ± 2.0 L min⁻¹, and power improved from 7.3 ± 0.9 to 6.6 ± 0.5 watts. Of the 2 failures, 1 went into polymorphic VT and died the day after t-PA. The other had ischemic stroke within 1 h of t-PA, followed by pump exchange, but had full recovery.

Conclusion: Using t-PA is a viable treatment option for LVAD thrombosis, but significant complications still remain a limitation.

Disclosure of Interest: None declared.

OR060

Application of a microfluidic flow-based thrombosis assay for post-operative pediatric cardiovascular surgery patients

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Background: Systemic-to-pulmonary shunts are surgically placed conduits that provide a stable source of pulmonary blood flow in infants with single ventricle physiology. The high-shear environment inside the shunt can activate platelets and cause thrombosis. Shunt obstruction is a significant cause of morbidity and mortality in these patients; therefore the infants are given prophylactic aspirin to prevent thrombosis. Current platelet function assays (whole blood aggregometry, WBA) don't incorporate shear and may underestimate platelet activity. Platelet function tests which incorporate physiologic shear may better predict the risk of shunt thrombosis

Aims: Determine whether a microfluidic thrombosis (MT) assay can detect a change in whole blood platelet function in aspirin-treated infants following shunt placement. Compare the kinetics of thrombus formation obtained from the MT assay vs. the WBA assay

Methods: Baseline samples from 5 infants were obtained before, 24 h, and 48 h post aspirin therapy. MT and WBA assays were performed on blood samples with and without the addition of exogenous aspirin. Lag time, slope, maximum amplitude, and area under curve (AUC) were analyzed

Results: The MT assay detected a larger range in lag times and slope. The WBA assay detected a larger range in maximum amplitude and AUC. The addition of exogenous aspirin uniformly increased lag time, and decreased slope, maximum amplitude, and AUC. After aspirin administration in the pediatric intensive care unit, two patterns emerged: 3 patients displayed a uniform increase in thrombosis throughout the assay and 2 patients displayed an accelerated initial increase to their maximum intensities followed by minimal changes in thrombotic activity

Conclusion: The MT assay detects a difference in platelet function in post-operative patients before and after treatment with aspirin. The MT assay may have potential to differentiate between thrombotic states in these patients. Larger studies are necessary to determine clinical significance

Disclosure of Interest: None declared.

NOACs in clinical practice

OR061

Adherence to anticoagulant treatment with dabigatran and rivaroxaban in a real-world setting

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Background: The non-vitamin K antagonist oral anticoagulants (NOACs) are effective in stroke prevention in non-valvular atrial fibrillation and in the treatment of venous thromboembolism (VTE). Although adherence was excellent in the clinical trials with NOACs, there is scarce data on this in clinical practice.

Aims: To compare adherence levels between the one-dose regimen of rivaroxaban and the two-dose regimen of dabigatran among patients in different clinical settings.

Methods: In a prospective cohort study data was collected on patients treated with dabigatran and rivaroxaban for at least 3 months at four different centers (two cardiology clinics, one family medicine clinic, and one anticoagulation clinic) in Canada and Sweden. Information on dabigatran and rivaroxaban prescriptions and refills was obtained from pharmacies, from the time point of the first prescription by any physician. We calculated the adherence from pharmacy refills.

Results: Data on 474 consecutive patients (205 on dabigatran, 269 on rivaroxaban) was collected from the participating centers (49% female, median age 73 years, range: 36–95), of which 97% had atrial fibrillation, 3% had VTE. There were no significant differences between the two treatment groups in age or sex. Patients on dabigatran and rivaroxaban were followed for a median (min-max) of 382 (98–1448) and 449 (95–908) days, respectively (p-value 0.16). The median (interquartile range) estimated adherence was 100% (97–100) on dabigatran and 100% (100–100) on rivaroxaban with 93% and 96% of patients, achieving adherence levels higher than 80%. None of the latter comparisons was statistically significant.

Conclusion: Our study shows high estimated adherence levels to dabigatran and rivaroxaban in clinical practice. There was no significant difference in the adherence level between the two-dose regimen of dabigatran and the one-dose regimen of rivaroxaban.

Disclosure of Interest: F. Al Khalili: None Declared, D. Suryanarayan: None Declared, C. Lindström Employee of: Boehringer Ingelheim, S. Schulman: None Declared, A. Majeed: None Declared

OR062

The impact of the introduction of dabigatran on anticoagulant management in New Zealand

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Background: In New Zealand (NZ), dabigatran became available on 1st July 2011. It was expected that it would lead to a reduction in the number of patients taking warfarin but an increase in the total number of patients on anticoagulants for atrial fibrillation (AF).

Aims: To audit warfarin usage and the growth of dabigatran in NZ since July 2011.

Methods: The change in anticoagulant use was evaluated from (1) prescribing data collected from the National Pharmaceutical database (NPD) and (2) using INR data from a cohort of patients on warfarin managed in the Central North Island of NZ (pop. 310,000). Data were collected from 1/4/11 to 30/9/14. Ethics approval was obtained.

Results: Before July 2011, ~7000 INR tests were performed per month on ~3400 patients in the central region of NZ. During the first 3 months following the introduction of dabigatran the INR test rate dropped to 6300/mth on ~3000 patients and plateaued at this rate over the next 30mths. The proportion of patients with AF on warfarin fell from 64% to 59% and median age increased from 76.8 to 78.4 yrs over 30 months. Data from the NPD showed ~21,000 prescriptions/month were issued for warfarin before July 2011 for ~42,000 patients. This dropped to ~17,000 prescriptions for ~38,000 patients within 3 months and has remained constant since. The number of patients starting warfarin in NZ fell from 800 to 650/mth over 3 yrs. Since July 2011 the number of patients on dabigatran has grown steadily; within 3 months 8000 patients were taking dabigatran and this has gone up to ~16,000; ~600 start and ~300 stop dabigatran each month.

Conclusion: The introduction of dabigatran led to an initial 12% fall in the number of patients taking warfarin. The stable numbers since with an increased median age suggests that established patients remain on warfarin with a small number changing to dabigatran. The majority of dabigatran patients are new to anticoagulants. The total number of patients on anticoagulants in NZ has increased 30% (42,000 to 55,000) mainly for AF.

Disclosure of Interest: P. Harper Shareholder of: INR Online Ltd (warfarin Management software), D. Pollock: None Declared, L. Chen: None Declared, S. C. Fong: None declared.

OR063

Real-world rivaroxaban levels from King's college hospital

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Background: Routine monitoring is not recommended for direct-acting anticoagulants, however as clinical experience grows there are circumstances in which it is desirable to measure drug levels: in patients with bleeding or recurrent thrombosis; suboptimal renal function; and, where absorption is in question. In considering the relevance of rivaroxaban levels for a given time post-dose, a peak and trough approach has been proposed. Currently there is no available data on how drug levels correlate with clinical outcomes.

Aims: To describe *real-world* rivaroxaban levels from patient's at King College Hospital.

Methods: Rivaroxaban levels taken from patients treated for all indications in 373 samples were evaluated. All were performed between March 2013 and September 2014. Drug level analysis was using a calibrated anti-Xa assay. All patients were on once daily dosing regimens.

Results: Mean values showed statistically significant correlation between time from dose and drug level using Spearman's one-tailed test ($P < 0.01$) for grouped quartiles of known sample times: 208.65 ng L⁻¹ at < 3 h ($n = 98$), 219.66 ng L⁻¹ at 3–12 h ($n = 93$), 114.45 ng L⁻¹ between 12–19 h ($n = 93$) and 73.48 ng L⁻¹ after > 19 h ($n = 89$). Taken alone, respective mean levels for the 15 mg and 20 mg dosing regimens were similar at peak, 196.56 ng L⁻¹ (SD = 117.41) and 213.82 ng L⁻¹ (SD = 137.95) and at trough, 93.79 ng L⁻¹ (SD = 70.19) and 64.18 ng L⁻¹ (SD = 64.01). Wide standard deviations demonstrate significant interindividual variability in rivaroxaban levels at given time from dose.

Conclusion: Mean rivaroxaban value by time since dose show relatively predictable levels with 15 mg and 20 mg dosing in real-world patients. Our results compare favourably with the peak of 223 ng L⁻¹ (range 160–360) proposed by original pharmacokinetic studies of rivaroxaban and given as the reference range for levels in 2014 BCSH guidelines. Work should now focus on validating how the current recommended targets translate to clinical outcomes.

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OR064

Prospective survey of adverse effects of non-vitamin K oral anticoagulants in two UK hospital trusts

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Background: Non vitamin K oral anticoagulants (NOACS) have increased in use over the last few years in the UK. Three are currently available: Apixaban (A), Rivaroxaban (R) and Dabigatran (D). As they are still new to the UK market, we prospectively monitor the side-effect profile at two hospital trusts.

Aims: The aims were to determine the percentage, type and duration of side-effects experienced by patients on NOACS by prospective monitoring of 'real world' data.

Methods: Data was recorded at 1–2 weeks and 3–6 months after starting a NOAC. The data recorded included the following: age, gender, indication, type/duration of adverse effect, and if continued.

Results: A total of 493 patients were included. The average age was 71 (range 20–96). The male:female ratio was equal with 246 females and 247 males. 346 patients were on a NOAC for stroke prevention in atrial fibrillation, and the remainder for venous thrombo-embolism (VTE). Side-effects were reported in 20% on A, 31% on R and in 40% on D. Gastro-intestinal disturbance occurred in 6.5% on A, 13% on R and in 24% on D. Both fatigue and headache occurred in about 5% across each group, and dizziness in 2.7% on A, 6% on R and 11% on D. Minor bleeding was common in all groups but did not cause patients to discontinue. Major bleeding was very rare, with one episode in A and R groups. Menorrhagia was reported more commonly in the R group, but this was likely due to use of R preferentially in younger patients for VTE treatment. Patients on D appeared to be more likely to have multiple side-effects. The symptoms of headache and dizziness were often temporary, and resolved if patients continued.

Conclusion: In conclusion, side-effects were common, but many were transitory and patients were able to continue. apixaban had the best profile across all side-effects, and dabigatran the worst, the latter often causing multiple side-effects in individuals affected. Major bleeding

events were very rare with all the drugs, despite the largely elderly population.

Disclosure of Interest: None declared.

OR065

Dabigatran but not rivaroxaban or apixaban reduces fibrinolytic resistance in patients with atrial fibrillation

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Background: Most anticoagulants stimulate fibrinolysis *in vitro* through TAFI-dependent and TAFI-independent mechanisms.

Aims: We evaluated the effect of dabigatran, rivaroxaban and apixaban treatment on plasma fibrinolysis in patients with non-valvular atrial fibrillation.

Methods: Three groups of 21–25 patients, receiving therapeutic doses of dabigatran etexilate, rivaroxaban or apixaban were studied. Plasma was obtained in the morning just before (*trough*) and 2 h after drug intake (*peak*). Median concentrations (ng mL⁻¹) at *trough* and *peak* were, respectively, 58 and 152 for dabigatran, 26 and 194 for rivaroxaban, and 115 and 240 for apixaban.

Results: Fibrinolytic resistance, measured as the lysis time of plasma clots exposed to 30 ng mL⁻¹ t-PA, was significantly lower in *peak* than in *trough* samples in dabigatran group ($P = 0.0001$) but not in rivaroxaban and apixaban groups ($P > 0.15$). Accordingly, drug concentration correlated with lysis time in dabigatran group only ($\rho = 0.55$, $P = 0.0002$). The median lysis time was shorter in dabigatran group as compared to the other two groups, both at *trough* ($P = 0.05$) and *peak* ($P = 0.0002$). This difference, however, disappeared ($P > 0.5$) if the fibrinolytic assay was performed in the presence of PTCl (TAFIa inhibitor), suggesting that the profibrinolytic effect of dabigatran treatment was largely TAFI-mediated. Thrombin generation (ETP) and TAFI activation were markedly lower in *peak* than in *trough* samples in all three groups ($P \leq 0.0001$). However, TAFIa levels in *trough* and *peak* samples were significantly lower in dabigatran group than in rivaroxaban and apixaban groups ($P < 0.001$), indicating a stronger inhibition of thrombin-mediated TAFI activation by dabigatran.

Conclusion: Dabigatran treatment reduces fibrinolytic resistance because, contrary to rivaroxaban and apixaban, it is able to decrease TAFIa generation below the threshold level required to halt the fibrinolytic process. Fibrinolysis stimulation may contribute to the anti-thrombotic activity of dabigatran.

Disclosure of Interest: None declared.

Management of bleeding

OR066

Role of tranexamic acid in reducing bleeding episodes and requirement of platelet support in patients with aplastic anemia

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Background: Patients with aplastic anemia require frequent admissions and suffer both mortality and morbidity on account of bleeds. Tranexamic acid has been found to be useful in patients with acute leuke-

mia undergoing chemotherapy. We studied its beneficial effects in patients with aplastic anemia.

Aims: To study the benefit of adding oral tranexamic acid as prophylaxis in patients with aplastic anemia.

Methods: We enrolled 80 patients > 18 years with acquired aplastic anemia. Group 1 received oral tranexamic prophylaxis at 500 mg TDS throughout the study period of 3 months and group 2 patients received tranexamic acid along with platelet support only during bleeding episodes. The student test/Mann Whitney test was applied to compare quantitative data between the two groups. Chi square test / Fisher exact test was applied to compare qualitative data. *P* value < 0.05 was considered as significant.

Results: Of the 80 patients enrolled, 6 died and 10 were lost to followup. The baseline data in group 1 and group 2 was as follows: median age 20 years (range 18–55) and 23 years (range 19–55), 28 and 33 males respectively, median total leukocyte counts 2000/mm³ and 1800/mm³, media platelet counts 9000/mm³ in both groups, severe aplastic anemia 35 and 36, very severe aplastics 5 and 4 respectively. The average requirement of pooled platelets in group 1 during study period was 14.06 ± 5.29 units and in group 2 it was 11.55 ± 6.03 units. The average requirement of packed RBCs was 10.61 ± 2.18 units in group 1 and 10.91 ± 2.40 units in group 2 patients. The average numbers of major bleeding episodes were 0.18 ± 0.39 in group 1 and 0.40 ± 0.50 were in group 2 patients and average numbers of minor bleeding episodes were 3.18 ± 1.74 in group 1 patients and 4.13 ± 1.76 was in group 2 patients. These differences were not statistically different.

Conclusion: Tranexamic acid did not reduce the bleeding episodes or requirement of blood component support in this cohort of patients with aplastic anemia.

Disclosure of Interest: None declared.

OR067

Effects of idarucizumab, given as a split dose, in a blunt liver double trauma model in pigs receiving dabigatran

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Background: Reversal of dabigatran with idarucizumab together with other resuscitation measures may become first line treatment in bleeding, traumatized patients on dabigatran anticoagulation.

Aims: This study tested the effect of the antibody fragment (Fab), idarucizumab, given once or as a split dose, on blood loss (BL) and coagulation after double trauma using a blunt liver injury in pigs anticoagulated with dabigatran.

Methods: Dabigatran etexilate (30 mg kg⁻¹ bid) was given to 18 male pigs for 3 days after ethical approval. On day 4, pigs were anesthetized and given a dabigatran infusion before blunt liver injury. Animals were randomized to receive, 60 + 0, 60 + 60 or 120 + 0 mg kg⁻¹ idarucizumab 1 h apart (120 mg kg⁻¹ Fab is equimolar to the dabigatran dose). Prior to the 2nd dose, a 2nd liver injury was made on a different lobe. BL, hemodynamic and coagulation parameters were monitored for 5 h or until death.

Results: Dabigatran levels were 1128 ± 160 ng mL⁻¹ prior to the 1st trauma. BL was ~800 mL 12 min after trauma but before treatment with 60 + 0, 60 + 60 or 120 + 0 Fab and similar among groups (*P* > 0.05). BL increased from ~800 mL to 990 ± 109 mL, 988 ± 84 mL or 964 ± 75 mL 1 h after treatment, respectively (*P* > 0.05 between groups). Treating with vehicle after the 2nd injury (60 + 0) resulted in total BL of 4167 ± 550 mL with 83% mortality

(*P* < 0.001 vs other groups). 120 + 0 Fab resulted in total BL of 1659 ± 346 mL and 100% survival 4 h after a 2nd injury. Splitting the Fab dose (60 + 60) resulted in a comparable BL (1426 ± 106 mL) and also 100% survival. Dabigatran anticoagulation, measured as dTT, aPTT or ECT was partially reversed after 60 + 0, but completely reversed with 60 + 60 and 120 + 0.

Conclusion: Idarucizumab rapidly reduced continuous bleeding induced by 2 traumatic injuries in animals under dabigatran anticoagulation and improved survival in this lethal porcine model. Equimolar inhibition of dabigatran resulted in anticoagulation reversal and restored hemostasis, regardless of whether given as a single or split dose.

Disclosure of Interest: M. Honickel: None Declared, J. van Ryn Employee of: Boehringer Ingelheim, R. Rossaint Consultant for: CSL Behring, Novo Nordisk, H. ten Cate Grant/Research Support from: Bayer Healthcare, Leo Pharma, Boehringer Ingelheim, Pfizer, Consultant for: Bayer Healthcare, Boehringer Ingelheim, H. Spronk Grant/Research Support from: Boehringer Ingelheim, Consultant for: Bayer Healthcare, O. Grottke Grant/Research Support from: CSL Behring, Novo Nordisk, Biotest, Boehringer Ingelheim, Consultant for: Bayer Healthcare, Portola, Boehringer Ingelheim.

OR068

Hemodialysis for the treatment of dabigatran-associated bleeding: a systematic review of the literature

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Background: Dabigatran, a direct thrombin inhibitor, is effective for the treatment of venous thromboembolism and prevention of stroke and systemic embolism from atrial fibrillation. The most effective means of reversing the anticoagulant effect of dabigatran in patients who have bleeding complications is unclear. We describe a case and review the literature assessing the efficacy of hemodialysis for the elimination of dabigatran.

Aims: To evaluate the efficacy of hemodialysis for dabigatran removal in patients with bleeding.

Methods: We performed literature search of MEDLINE and EMBASE up to October 2014. Articles were selected if the patients presented with dabigatran-associated bleeding, underwent hemodialysis for dabigatran removal and reported the effect on bleeding.

Results: The search yielded 135 citations; 15 studies involving 20 unique cases were included in a qualitative systematic review. Median patient age was 77.5 years (range: 58–94), 9 (45%) were female and 90% of patients received dabigatran for atrial fibrillation. All patients had renal impairment (creatinine clearance < 80 mL min⁻¹). Fourteen patients (70%) underwent intermittent hemodialysis, 5 patients (25%) underwent continuous renal replacement therapy (CRRT) and 1 patient underwent both intermittent hemodialysis and CRRT. Following hemodialysis, there were significant reductions in dabigatran levels (*P*-value = 0.01). Rebound of the dabigatran level was reported in 6 of 8 patients following cessation of dialysis. Hemostasis was reportedly achieved in 14 patients (70%) and 4 patients (20%) died due to bleeding.

Conclusion: Hemodialysis appears to be effective in reducing the anticoagulant effect of dabigatran in patients with dabigatran-associated bleeding but a rebound in levels may be seen following withdrawal of dialysis. This finding suggests that prolonged dialysis exposure may be more effective than shorter courses.

Disclosure of Interest: None declared.

OR069

Optimal timing of warfarin resumption after warfarin related upper gastrointestinal bleeding

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Background: The outcome and optimal timing of warfarin resumption after warfarin-related upper gastrointestinal bleeding (WUGIB) is not well studied.

Aims: To describe the risk of recurrent gastrointestinal (GI) bleeding or thromboembolic events in relation to warfarin resumption status after WUGIB.

Methods: Retrospective review of medical journals of patients who suffered WUGIB between 2004 and 2010 in one center in Canada and three centers in Sweden. Data on the timing and occurrence of recurrent GI bleeding or thromboembolic events before and after warfarin resumption were collected.

Results: In total, 207 patients with WUGIB were included in the study (median age 77 years, women 37%). The majority of patients had cardiac indication for warfarin treatment (atrial fibrillation 63%, mechanical heart valves 14%). Patients were followed for a median (interquartile range [IQR]) of 124 (45–224) weeks, during which 27% suffered recurrent GI bleeding. Fifty percent of the patients died during follow-up after a median (IQR) of 84 (18–152) weeks. Warfarin was resumed in 58% of the patients after a median (IQR) of 1.0 (0.2–4.0) weeks. Warfarin resumption earlier than 3 weeks after WUGIB was associated with a significant increase in the risk of recurrent GI bleeding (log-rank p-value 0.014), with no such difference seen when warfarin was resumed after 3 weeks (log rank *P*-value = 0.52). Four patients with atrial fibrillation suffered thromboembolic events within 3 weeks after WUGIB as compared to 17 events occurring after 3 weeks (including 5 events in patients who resumed warfarin, all occurring after 85 weeks after warfarin resumption).

Conclusion: WUGIB is associated with high recurrence and mortality rates. Warfarin resumption later than 3 weeks after WUGIB seems to be associated with lowest thromboembolism/ GI bleeding recurrence rates.

Disclosure of Interest: None declared.

OR070

Fibrinogen concentrate improves fibrin network structure during orthotopic liver transplantation

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Background: Optimal coagulation management remains a challenge in orthotopic liver transplantation (OLT) due to impaired synthesis and/or catabolism of hemostatic proteins and decreased platelet counts in patients with liver disease. Fibrinogen concentrates are increasingly used, but the use of these concentrates to correct the perioperative bleeding is based on empirical evidence and arbitrary thresholds of fibrinogen levels. Studies on clot structure are lacking.

Aims: We aimed to determine the haemostatic efficacy of fibrinogen concentrate in correcting fibrin structure.

Methods: Plasma samples were obtained at the start of surgery, in the anhepatic phase, after reperfusion, and at the end of surgery. Plasma

samples of 15 patients and 15 controls were spiked with 1 g L⁻¹ of fibrinogen concentrate. Fibrin polymerization was monitored by changes in turbidity. Fiber density was determined by measuring fibers crossing a line using confocal microscopy. The average pore size of the fibrin clot (Ks) was assayed in permeation studies.

Results: Fibrin polymerization was significantly decreased at the start of the surgery compared with controls and decreased even further during surgery. Addition of fibrinogen increased polymerization significantly for all time points, but did not normalize it. Fibrin density was significantly reduced after reperfusion compared with fiber density at the start of surgery and with healthy controls. Fibrin density improved significantly after addition of fibrinogen. The severely impaired polymerization and decreased density after reperfusion was accompanied with significantly increased permeability of the clot compared with the start of surgery and controls. Addition of fibrinogen completely restored the permeability of the clots at the start of surgery and after reperfusion.

Conclusion: *Ex vivo* addition of fibrinogen concentrate during OLT substantially improved structural properties of the fibrin clot, which, particularly after reperfusion, displayed hypocoagulable features.

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Venous thromboembolism epidemiology – I

OR071

Impact of incident myocardial infarction on future risk of venous thromboembolism

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Background: Recent studies have demonstrated an association between venous thromboembolism (VTE) and arterial thrombotic diseases. Results from a large registry-based cohort study suggest that the opposite interrelation exists between myocardial infarction (MI) and future risk of VTE. Data from registry-based studies should, however, be interpreted with caution due to lack of validation of outcomes and ability to adjust for confounders.

Aims: To study the association between incident MI and VTE in a large prospective cohort recruited from a general population.

Methods: Participants from the Tromsø study (*n* = 29 506) without a previous history of VTE and MI were followed from baseline to the date of an incident VTE, death, migration or the end of the study period. Cox regression models with age as time scale and MI as a time-dependent variable were used to calculate hazard ratios (HR) adjusted for sex, BMI, diabetes, smoking, blood pressure, HDL-cholesterol, physical activity and education. The study was approved by the regional ethical committee and all subjects gave their informed written consent.

Results: There were 1924 cases of first-time MI with 48 subsequent VTEs during a median follow-up of 15.7 years. MI was associated

with a 51% increased risk of VTE (HR 1.51; 95% CI, 1.08–2.10) and 70% increased risk of pulmonary embolism (PE) (HR 1.70, 95% CI 1.05–2.75), but there was no significant association with deep vein thrombosis (DVT). MI explained 6.2% of the PEs in the population (population attributable risk) and 78.5% of the PE risk in MI patients (attributable risk). The highest risk estimates were observed during the first sixth months after MI (HR 8.49; 95% CI, 4.00–18.77).

Conclusion: Our findings indicate that MI is associated with increased risk of VTE independent of traditional cardiovascular risk factors. The risk estimates was particularly high for PE, and 6.2% of the PEs in the population was attributed to MI.

Disclosure of Interest: None declared.

OR072

Development and validation of a patient self-administered villalta scale for patient self-assessment of the post-thrombotic syndrome

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Background: Post-thrombotic syndrome (PTS) is a long-term complication of deep vein thrombosis (DVT). The Villalta scale is the recommended tool for diagnosing PTS, but requires a clinician's assessment in addition to patient self-assessment.

Aims: To validate a self-administered tool for patient reporting of leg symptoms and signs as a mean to assess the post-thrombotic syndrome.

Methods: The first part of the study involved validation of a simple form for patient-reported Villalta (PRV1). PTS diagnosed using PRV1 scale showed low concordance with PTS diagnosed by the original Villalta scale. Consequently, we developed a visually-assisted form for patients' reporting of signs and symptoms of the Villalta scale (PRV2), which was based on a visual guide developed by Kahn et al. (J Thromb Haemost 2009; 7: 879). The validity of PRV1 and PRV2 was assessed in patients diagnosed with DVT between 2004 and 2012. Mean time from DVT to inclusion was 5.1 and 4.3 years for PRV1 and PRV2, respectively. Patients were requested to complete the PRV form before a scheduled visit. At the visit PTS was diagnosed by the original Villalta scale, which served as the reference method.

Results: PRV1 showed only moderate agreement for diagnosing PTS compared to the original Villalta scale (kappa agreement 0.61, 95% CI 0.49–0.73). Among 94 patients (median age 61 years, IQR 51–70 years) who participated in the validation of PRV2, PTS was diagnosed in 54 (57%) patients according to original Villalta scale and 61 (65%) by PRV2. The kappa agreement between the two assessments was 0.82 (95% CI 0.70–0.94; very good agreement). The sensitivity of PRV2 was 99% and the specificity was 83%.

Conclusion: The visually assisted form for PRV is a valid and sensitive tool for diagnosing PTS. Such a tool could be applied in further clinical studies of PTS, making studies less resource demanding by reducing the need for in-person clinic visits.

Disclosure of Interest: None declared.

OR073

Outcomes from the national venous thromboembolism prevention programme in England

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Background: The National Venous Thromboembolism (VTE) Prevention Programme was launched in England in 2010 to reduce avoidable death, disability and ill health as a consequence of hospital-associated thrombosis (HAT). The programme encompassed mandatory VTE risk assessment (RA) using a national RA tool, national guidance on thromboprophylaxis and requirements for audit and root cause analysis. Supporting resources include the national VTE exemplar network, a national VTE prevention website, e-learning and patient information.

Aims: To determine the impact of the National VTE Prevention Programme.

Methods: VTE RA rates were retrieved from the UNIFY database and expenditure data on prophylactic heparin from NHS information centre. Hospital Episode Statistics were examined to determine the incidence of HAT defined as any VTE occurring within 90 days of discharge.

Results: VTE RA rates increased significantly from 46.7% (July 2010) to 91.3% (November 2011) and now maintained at 96%. Weighted hospital expenditure on prophylactic heparin increased by 23.8% from 2009 to 2011 and then stabilised. The VTE e-learning module has been completed by > 20000 individuals with 1054 website visits/month. The incidence of HAT overall remains unchanged. Within elective admissions, there was a reduction in the incidence of deep vein thrombosis (DVT), particularly in high VTE risk patients (18.2 vs 15.6 per 1000 readmissions in 2006 vs 2013 respectively) with marked reduction in high risk surgical patients (22.1 vs 17.2), and a reduction in mortality due to VTE from 3.5 to 2.6. Of note, within low VTE risk groups, the incidence of VTE increased over this time period suggesting increased diagnostic rates, which may be a reflection of raised VTE awareness. Financial incentives driving improved coding may be an additional contributor.

Conclusion: The National VTE Prevention Programme has significantly improved VTE RA and prescription of thromboprophylaxis with a reduction in DVT incidence associated with elective hospital admissions.

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OR074

Risk stratification of patients with acute symptomatic pulmonary embolism based on presence or absence of deep vein thrombosis: systematic review and meta-analysis

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Background: The prognostic significance of concomitant deep vein thrombosis (DVT) in patients with acute pulmonary embolism (PE) lacks clarity.

Aims: In patients with acute symptomatic PE, we compared the risk of death in those with and those without concomitant DVT.

Methods: We performed a systematic review and a meta-analysis of studies in patients with acute PE to assess the prognostic value of concomitant DVT for 30-day all-cause mortality and 90-day PE-related adverse outcomes (PE-related death or non fatal venous thromboembolic recurrences). We conducted unrestricted searches of Medline and Embase from 1980 through September 30, 2014 and used the terms "deep vein thrombosis", "pulmonary embolism", and "prognos*". We used a random-effects model to pool study results; funnel-plot inspection to evaluate for publication bias; and I² testing to assess for heterogeneity.

Results: The analyses included data from 10 studies (8859 patients). Two hundred seventy two of 4379 patients with concomitant DVT died (6.2%; 95% confidence interval [CI], 5.5 to 7.0) compared with 133 of 3489 without DVT (3.8%; 95% CI, 3.2 to 4.5). Concomitant DVT had a significant association with 30-day all-cause mortality in all patients (7 studies; odds ratio [OR], 1.9; 95% CI, 1.5 to 2.4; I² = 0%) and in the subgroup of hemodynamically stable patients (2 studies; OR, 1.8; 95% CI, 1.2 to 2.8; I² = 0%). Concomitant DVT was not significantly associated with 90-day PE-related adverse outcomes (5 studies; OR, 1.6; 95% CI, 0.8 to 3.4; I² = 75%).

Conclusion: In patients diagnosed with acute symptomatic PE, whether or not stratified by hemodynamic stability, concomitant DVT was significantly associated with an increased risk of death.

Disclosure of Interest: None declared.

OR075

Regular physical activity and future risk of myocardial infarction and venous thrombosis – the tromsø study

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Background: Arterial and venous thrombosis are reported to be inter-related, potentially through shared risk factors. Physical activity is

known to have a beneficial impact on the risk of myocardial infarction (MI), whereas the relationship between physical activity and venous thromboembolism (VTE) is less clear.

Aims: To assess and compare the associations between the weekly duration of high-intensity physical activity and risk of MI and VTE, respectively, in a cohort recruited from the general population.

Methods: The study included 25980 subjects enrolled in the 4th (1994/95) survey of the Tromsø study. Self-reported weekly hours of hard physical activity (activity associated with sweat/breathlessness) were assessed in four categories ("none", "< 1 h", "1–2 h" and "3 or more hours") at baseline, and 5296 subjects were re-measured at the fifth survey (2001/02). All validated incident events of MI and VTE were recorded up to study end (Dec 2010). Cox-regression models with physical activity as a time-varying exposure were used to calculate hazard ratios for MI and VTE adjusted for age, sex and BMI.

Results: There were 1846 MIs and 576 VTEs during a median of 15.8 years of follow-up. Increasing hours of physical activity were associated with reduced risk of MI (p for trend across categories < 0.001), and subjects who reported ≥ 3 h/week had 35% lower risk than those who reported no activity (HR 0.75, 95% CI: 0.62–0.89). There was no gradient across increasing categories of physical activity and risk of VTE (p for trend 0.7) and the HR for ≥ 3 h/week vs. no activity was 1.00 (95% CI: 0.73–1.37). However, subjects who reported < 1 h/week of physical activity had a 22% lower risk of VTE (HR 0.78, 95% CI: 0.61–1.00).

Conclusion: Physical activity had differential impact on MI and VTE risks. Moderate amounts of physical activity protected against VTE, whereas an inverse dose-response relation appeared between the weekly amount of physical activity and MI risk.

Disclosure of Interest: None declared.

Thrombophilia

OR076

Antithrombin dublin (P.VAL30GLU): a polymorphism with moderate risk of thrombosis that causes a transient antithrombin deficiency by intracellular folding into the latent conformation

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Background: The key role of antithrombin (AT) in hemostasis explains the high risk of thrombosis associated with its deficiency but makes difficult to understand the low incidence of AT deficiency among patients with venous thrombosis.

Aims: To find out and characterize transient defects of AT that might be underdiagnosed by functional methods.

Methods: *SERPINC1*, the gene encoding AT, was sequenced in 162 subjects with a positive test for AT deficiency. Recombinant AT was expressed in HEK-EBNA cells. Biochemical (Edman's sequencing, calorimetry and electrophoresis) and functional analysis of purified proteins were also done. Thrombotic relevance was tested in a case control study including 1593 patients with venous thrombosis and 2592 controls from 2 populations.

Results: p.Val30Glu, responsible for the Dublin variant, was identified in 7/162 cases, 2 with double heterozygosity. The 5 cases carrying only this mutation had AT activity within the normal range in the sample delivered to our laboratory. In our recombinant system this mutation did not significantly affect the secretion of the variant, which lacks the 2 amino-terminal residues as does the variant purified from carriers' plasma. However, both intracellular and secreted recombinant variant folded into a latent non-inhibitory conformation. Genotyping of p.Val30Glu revealed that it is a low prevalent polymorphism (0.3%) that moderately increased the risk of venous thrombosis (OR:2.7; 95% CI:1.04-6.96; $P = 0.02$) and identified one homozygous patient with an early thrombotic event.

Conclusion: p.Val30Glu is a new mild prothrombotic polymorphism that has transient functional consequences in AT. By affecting the cleavage of AT's signal peptide, the variant AT lacks the N-terminal dipeptide, a defect with no significant functional consequences in normal conditions, but that allows the intracellular folding into a latent conformation under certain conditions, with loss of function and increased risk of thrombosis.

Disclosure of Interest: None declared.

OR077

Age-dependent factor IX activity in association with the A/A-genotype of F9 G32023A -polymorphism (RS440051) and consequent risk of venous thromboembolism (VTE)

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Background: In animal experiments, an "age-related increase element" (AIE) in the factor 9 gene has been found to elevate factor IX activity with age (Kurachi et al. 2005).

Aims: We investigated whether there are genetic variants in the factor 9-associated age element in humans associated with a higher than proportional increase of factor IX activity with age, constituting a risk factor of VTE.

Methods: We recruited VTE patients ($n = 187$) and controls ($n = 200$) matched for age, sex and estrogen therapy and sequenced their AIE in the factor 9 gene. We then tested a larger cohort of female patients for one polymorphism found to be associated with VTE in females.

Results: We identified six polymorphisms in the AIE, one of which (genotype AA of F9 G32023A polymorphism [rs440051]) was associated with an age-dependent increased risk of VTE in females, but not in males (> 50 years of age: 15/167 [9.0%] vs. 8/222 controls [3.60%], OR 2.64 [95% CI 1.09-6.38]). In a multivariate model, factor IX activity increased significantly in the presence of genotype AA ($P = 0.033$) and with age ($P = 0.004$). Furthermore, the polymorphism showed a significant interaction ($P = 0.033$) with age leading to increased factor IX activity.

Conclusion: The genotype AA of F9 G32023A polymorphism (rs440051) is associated with an age-dependent increase in factor IX activity and risk of VTE in females. Our results may be explained by findings of a heterogeneous, age-dependent incomplete inactivation of the X chromosome in females.

Disclosure of Interest: None declared.

OR078

The incidence of VTE in asymptomatic carriers of a deficiency of at, PC OR PS: a prospective cohort study

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Background: Deficiency of antithrombin, protein C, and protein S are associated with an increased risk of venous thromboembolism.

Aims: The objective of this study was to prospectively assess the incidence of venous thromboembolism in non treated asymptomatic subjects with such a deficiency.

Methods: We conducted a prospective cohort study in asymptomatic family members of patients who presented with a venous thromboembolic event, a family history positive for venous thromboembolism and who were found to have a deficiency of antithrombin, protein C or protein S. No anticoagulant prophylaxis was given to the study participants, except during risk periods for venous thromboembolism (surgery, trauma, immobilization, pregnancy). All venous thromboembolic events were diagnosed by objective diagnostic tests.

Results: A total of 252 individual carriers of thrombophilia were included in the study and compared with 249 asymptomatic family members no carriers of thrombophilia. A total of 3196 patient observation years was obtained in the first group and 4099 patient observation years in the second group. A total of 68 venous thromboembolic events occurred, resulting in an annual incidence of 2.1% (95% CI, 1.6 to 2.7) for the 3 deficiencies combined. Thirty-nine of these events occurred spontaneously, resulting in an annual incidence of spontaneous venous thromboembolism of 1.2% (95% CI, 0.9 to 1.7). No spontaneous events occurred in the control group. OR 50.0% (95% CI 6.9 to 364) A total of 99 risk periods occurred in the carriers group, during which 29 subjects developed a venous thromboembolic event (21.2%; 95%CI, 13.1 to 32.4).

Conclusion: We conclude that during risk periods for venous thromboembolism, adequate prophylaxis is necessary. Further studies are needed to assess the adequate dose and the optimal duration. The use of continuous anticoagulant prophylaxis seems not warranted in asymptomatic individuals with a deficiency of antithrombin, protein C or protein S.

Disclosure of Interest: None declared.

OR079

Antithrombin heparin binding site deficiency: a challenging diagnosis of a not so benign thrombophilia

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Background: Hereditary antithrombin (AT) deficiency is a rare autosomal dominant disorder characterised by a decreased AT activity in plasma and a predisposition to recurrent venous thromboembolism (VTE). Thrombotic risk is thought to vary according to the subtype of deficiency, with Heparin Binding Site (HBS) deficiencies being the less thrombogenic.

Aims: This study aimed to investigate the genetic background of AT HBS deficiency, to assess sensitivity of commercial AT activity assays for HBS mutations and to study the associated clinical picture.

Methods: The study population consisted of 81 genetically confirmed HBS deficient patients. Plasma samples of 35 of these patients were used for the evaluation of 4 commercial activity assays (3 Xa-based and 1 IIa-based) in their ability to diagnose HBS deficiency. Mutation-specific prevalence of venous and arterial thrombosis and the contribu-

tion of additional thrombophilic risk factors were evaluated in the whole study population.

Results: Six different mutations were identified in the studied patients. In one patient, a novel mutation, p.Asn77His, was identified, a quite exceptional finding given the restricted number of causal mutations in AT HBS deficiency. Only one assay (Xa-based) showed 100% sensitivity for all HBS mutations. The others failed mainly in identifying patients with p.Pro73Leu and p.Arg79His mutations. The overall prevalence of VTE in the studied subjects, 36%, was higher than previously reported in patients with this subtype. Arterial thrombosis occurred in 19% of the patients, mainly in carriers of the p.Pro73Leu and p.Arg79His mutations. VTE occurred in 8 out of 10 patients with additional Factor V Leiden or PT G20210A mutation, justifying complete thrombophilia screening in these patients.

Conclusion: Our data proof the limited sensitivity of commercial assays in the identification of patients with AT HBS deficiency, while this subtype seems more prevalent and less benign than previously assumed.

Disclosure of Interest: None declared.

OR080

High neutrophil and basophil blood counts are associated with increased factor II plasma coagulant activity and may be predictors of mortality in patients with stable coronary artery disease

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Background: White blood cell (WBC) count is widely available in clinical practice and has been proposed to provide prognostic information in coronary artery disease (CAD), with elevated levels of WBC predicting adverse cardiovascular outcomes. Recently, neutrophil extracellular traps (NETs) have been demonstrated to be a scaffold and stimulus for thrombus formation.

Aims: To investigate i) the correlation between WBC counts and factor II plasma coagulant activity (FII:c), and ii) WBC prognostic significance in the setting of secondary prevention of CAD.

Methods: Within the framework of the angiographically-controlled Verona Heart Study, WBC count and FII:c were analyzed in 750 subjects (554 CAD and 196 CAD-free, 77.6% males, mean age 60.0 ± 10.5 years) not taking anticoagulant drugs. Moreover, WBC count was assessed as predictor of total and cardiovascular mortality in 823 stable CAD patients (80.2% males, mean age 61.8 ± 9.7 years). Subjects with overt leukocytosis ($> 10,000 \mu\text{L}^{-1}$) or leukopenia ($< 4000 \mu\text{L}^{-1}$) were excluded.

Results: Neutrophils ($\beta = 0.085$; $P = 0.021$) and basophils ($\beta = 0.073$; $P = 0.042$) were significant predictor of FII:c variability in a linear regression model adjusted for all blood cell counts, age, sex, hs-CRP, and CAD diagnosis. In the longitudinal study, after a median follow-up of 61 months, 160 (19.4%) subjects died, 107 (13%) of whom for cardiovascular causes. High levels of neutrophils, monocytes, eosinophils, and basophils were associated with an increased mortality rate in the CAD population. However, in multi-adjusted Cox regression models only neutrophils and basophils remained predictors of total [the highest vs. the lowest quartile HRs: 2.87 (1.54–5.34) and 1.71 (1.06–2.75), respectively] and cardiovascular mortality [HRs: 3.31 (1.42–7.71) and 1.85 (1.02–3.35), respectively].

Conclusion: High neutrophil and basophil blood counts are associated with an enhanced FII:c and may be independent predictors of total and cardiovascular mortality in patients with stable CAD.

Disclosure of Interest: None declared.

Megakaryocytes and thrombopoiesis I

OR081

Genetic ablation of trpm7 alters magnesium homeostasis and induces macrothrombocytopenia in mice

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Background: TRPM7 is a ubiquitously expressed bi-functional protein comprising a non-selective Mg^{2+} and Ca^{2+} channel segment (TRP) linked to a α -type serine/threonine protein kinase domain. Abnormal channel activity of TRPM7 has been shown to interfere with many cellular processes, such as cell adhesion, migration, proliferation and cell survival. The α -type serine/threonine protein kinase domain of TRPM7 phosphorylates annexin I and non-muscle myosin IIA heavy chain in mammalian cells, thus interfering with cytoskeletal rearrangements. The importance of the channel and kinase function of TRPM7 in megakaryopoiesis and platelet function is unknown.

Aims: We aimed to elucidate the role of TRPM7 in cation homeostasis of megakaryocytes (MKs) and platelets.

Methods: We analysed a MK- and platelet-specific TRPM7 knock-out mouse strain (*Trpm7*^{fl/fl-PF4Cre}). Mutant MKs and platelets were analyzed using a wide range of *in vitro* and *in vivo* assays.

Results: *Trpm7*^{fl/fl-PF4Cre} mice develop macrothrombocytopenia due to impaired platelet production by MKs. In line with this, increased MK number in the femur, abnormal ploidy and ultrastructure of the invaginated membrane system were observed. Ultrastructure of *Trpm7*^{fl/fl-PF4Cre} platelets showed aberrant organization of microtubules and abnormal distribution of organelles. *Trpm7*^{fl/fl-PF4Cre} platelets displayed abnormal Mg^{2+} and Ca^{2+} homeostasis in resting and activated states. In *Trpm7*^{fl/fl-PF4Cre} platelets, a markedly impaired α -granule secretion was observed upon stimulation of both ITAM- and GPCR-coupled receptors. Surprisingly, *Trpm7*^{fl/fl-PF4Cre} platelets showed enhanced aggregation responses and ATP release to GPCR agonists, indicating multiple roles of the channel and kinase activity of TRPM7 in platelet physiology.

Conclusion: These results suggest TRPM7 as a key regulator of Mg^{2+} homeostasis in platelets, and point to a possible involvement of intracellular hypomagnesaemia in platelet disorders.

Disclosure of Interest: None declared.

OR082

Of men and mice: divergence effect of HDAC6 on proplatelet formation

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Background: Pan HDAC inhibitors (HDACi) are used to treat malignancies. Unfortunately they can induce a profound thrombocytopenia, due to a defect in megakaryocyte (MK) differentiation and proplatelet formation (PPF). Selective HDAC6 inhibitors such as Tubastatin or ACY-1215 (Ricolinostat) are in development. ACY-1215 is currently in clinical trials and induced a minimal thrombocytopenia (Raje et al, *Blood* [ASH Annual Meeting Abstracts] 2012 120: 4061)

Aims: The aim of this study is to decipher the role HDAC6 during PPF.

Methods: CD34⁺ or Lin⁻ cells, ACY1215 and Tubastatine as well as sh HDAC6 and HDAC6 KO mice were used for this study.

Results: HDAC6 is an HDAC that shuttles between the cytoplasm and the nucleus and regulates a variety of cellular processes. Here we show that HDAC6 was exclusively localized in the cytoplasm of human MKs. Tubastatin A and ACY-1215 mediated inhibition and of 2 shRNAs against HDAC6 induced a strong decrease in PPF. The defect in PPF was related to an increase in p53 acetylation and in RhoA activity. Use of a p53 shRNA and a ROCK inhibitor, Y27632, rescued the HDAC6-induced PPF decrease. Surprisingly murine HDAC6 (mHDAC6) was found to be mainly nuclear and its knock down did not affect PPF *in vitro*, and *in vivo*. Furthermore HDAC6 KO mice displayed a normal platelet level. The human HDAC6 differs from murine HDAC6 by the presence of NES1 and a NES2 domain. By targeting these domains, we constructed a hHDAC6 mutant that was retained in the nucleus. In accordance to our previous results we demonstrate that wild type but not HDAC6 mutant, restored the PPF decrease after shHDAC6 treatment.

Conclusion: Together our results suggest that unlike mHDAC6, hHDAC6 is cytoplasmic and regulates PPF *in vitro* by regulating Rho activity and p53 acetylation. The fact that HDAC6 and pan HDACi have in common to inhibit PPF, and that only pan HDACi affect hematopoietic progenitors suggest that the profound thrombocytopenia induced by pan HDACi is a consequence of the hematopoietic progenitor toxicity.

Disclosure of Interest: None declared.

OR083

Glycoprotein VI interactions with collagen type I selectively inhibit proplatelet formation

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Background: Platelets express two major collagen receptors, the integrin $\alpha 2/\beta 1$ and GPVI. After vessel injury, collagen present in subendothelial tissue is exposed to platelets and induces their activation, mediated by Syk-derived signaling. Interestingly, collagen type I is known to inhibit proplatelet formation (PPF), while collagen IV is reported to support thrombopoiesis. The underlying receptors are poorly characterized.

Aims: We aimed to elucidate the interaction between different collagen types and their receptors on megakaryocytes to understand how platelet formation is orchestrated at the vascular niche.

Methods: We used MKs of mice lacking either collagen receptor GPVI or integrin $\alpha 2$ and performed proplatelet assays. In addition, we developed a system of mixed matrix proteins to study which matrix proteins and which receptors are involved. These results were corroborated by blocking these receptors with specific antibodies.

Results: Collagen I-mediated inhibition of PPF is selectively mediated by GPVI, while mice lacking integrin $\alpha 2$ were virtually unaffected. These results were phenocopied when GPVI was blocked by JAQ1 antibody or integrin $\alpha 2$ by LEB/B. Vascular niche proteins like collagen type IV and laminin-111 had no effect on PPF. Using mixed substrates we found that the inhibitory signal by GPVI is dominating. Unexpectedly, collagen-related peptide (CRP) that concentrates the relevant GPO-motif and which is a strong inductor of Syk-LAT signaling, cannot trigger this inhibition, while it competes for binding with collagen I. Also, we could not detect Syk phosphorylation of MKs plated on collagen I.

Conclusion: Our results show for the first time that the inhibitory effect on PPF is selectively mediated by GPVI. Adhesion assays indicate that MKs binding on collagens is mostly mediated by integrin $\alpha 2/\beta 1$ and that collagen IV at the vascular niche might thus compete with binding and finally displaces collagen I from MKs and allows the directed release of platelets into the blood.

Disclosure of Interest: None declared.

OR084

Rapid 'rupture'-type thrombopoiesis processes from bone marrow megakaryocyte in response to acute platelet needs is regulated by IL-1ALPHA

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Background: Blood platelets are generated in the bone marrow (BM) from their precursors, megakaryocytes (MK). Although we know that MKs produce platelets throughout life, precisely how platelets are produced *in vivo* remains uncertain.

Aims: We revealed the regulating mechanisms of acute platelet supply from bone marrow MK, and approached to unanswered questions: 'How large number of platelets are produced from limited numbers of MKs?'.

Methods: To clearly understand the nature of thrombopoiesis in BM MKs, we optimized an *in vivo* imaging technique based on two-photon microscopy.

Results: By visualizing living bone marrow *in vivo*, we observed that a second thrombopoietic process, 'rupture'-like MK fragmentation, can be ongoing simultaneously with previously identified 'proplatelet formation' in the same mouse BM. Short proplatelets predominated in the steady state, but highly elongated proplatelets were apparent when thrombopoietin (TPO) levels were high (e.g., after BM transplantation). Conversely, following blood loss, 5-FU administration, antibody-based platelet depletion or acute inflammation, there was accelerated release of larger platelets from mature MKs mediated by novel 'rupture' type MK behaviors. Rupture was regulated by the interleukin-1 (IL-1)alpha-type1 IL-1 receptor axis and MK apoptosis. The custom software analysis elucidated that the number of produced platelets from one MKs by 'rupture' is larger than that by proplatelet formations, and the platelets are released preferentially into vessel lumens. It is known that proper microtubule assembly is vital for proplatelet formations in TPO-stimulated MKs, but did not in IL-1alpha-stimulated MKs due to uncoordinated expression of alpha- and beta-tubulin.

Conclusion: These findings support the ideas that IL-1alpha acts acutely as a platelet releasing factor, coordinating with TPO to dynamically modulate the cellular programming of MKs that regulates platelet counts.

Disclosure of Interest: None declared.

OR085

Synthesis of marcks in the late stages of megakaryocyte maturation drives proplatelet formation

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Background: Thrombocytopenia is a major clinical problem. Megakaryocytes (MKs) generate platelets by extending long branching processes, proplatelets, into blood vessels. While platelet production has been studied extensively, very little is known about what regulates proplatelet formation.

Aims: Identify and study proteins and corresponding signaling pathways that power proplatelet formation. We hypothesize proteins that aid in cytoskeletal reorganization are upregulated during proplatelet formation.

Methods: To identify dynamically changing proteins, we compared the proteome and transcriptome of round v. proplatelet-producing MKs by 2D DIGE and polysome profiling. To study MARCKS in MKs, we used a fluorescent inhibitory peptide and confocal microscopy. We used MARCKS KO mice to uncover MARCKS's role in proplatelet formation.

Results: The MARCKS protein was up-regulated 3.4 and 5.7-fold in proplatelet-producing MKs in both 2D DIGE and polysome profiling, respectively. MARCKS is a PKC substrate that binds PIP₂ in its dephosphorylated form and regulates actin polymerization. In MKs, MARCKS is localized to the demarcation membrane, a membrane reservoir for proplatelet formation. To examine MARCKS in the PKC pathway, we treated MKs with PMA; proplatelet formation was attenuated 84%, suggesting dephosphorylated MARCKS promotes proplatelet formation. This is supported by data that MARCKS increased over 3-fold in proplatelet MKs while pMARCKS decreased 1.8-fold. Consistently, pMARCKS, pWASP and pARP2/3 were significantly decreased in MKs making proplatelets. MARCKS inhibition by peptide or using KO mice decreased proplatelet formation 53 and 48%, respectively.

Conclusion: MARCKS expression and signaling in MKs is novel. We propose MARCKS binds PIP₂ in the demarcation membrane, obstructing PKC signaling that inhibits proplatelet formation. A better understanding of mechanisms regulating proplatelet formation will result in targeted therapeutics to increase platelet counts *in vivo* and make platelets *ex vivo* for transfusion.

Disclosure of Interest: None declared.

Von Willebrand Disease

OR086

Detection of large exonic and intergenic deletions in the VWF locus of VWD patients using array comparative genomic hybridisation (ACGH)

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Background: von Willebrand disease (VWD) is caused by mutations in von Willebrand factor (VWF), a large multimeric glycoprotein, essential for platelet dependent primary haemostasis and binding and transporting factor VIII. Mutations in the VWF gene (*VWF*) result in qualitatively defective (type 2), or quantitatively deficient (types 1 and 3) VWD. In type 1 VWD, ~35% of patients have no causative *VWF* mutation following exon and intron-exon boundary sequencing. Copy number variation (CNV) consisting of large exonic deletions/duplications within *VWF* has been reported, but little is known about the pathogenicity of CNV in *VWF* non-coding regions. We hypothesise that deep intronic and flanking untranslated region (UTR) CNV contribute to VWD.

Aims: To use aCGH to identify novel CNV across the *VWF* locus, including introns, 5' and 3' UTR that may contribute to VWD pathogenesis.

Methods: Custom 8x15K and 8x60K microarrays were designed (Agilent Technologies) containing ~1800 probes spanning the *VWF* locus. 18 patients with known CNV were selected for validation including whole-gene deletions, 6 pseudogene region deletions (exons 28, 32–34, 33–34), deletions of exons 3, 4–5, 17–18 and a duplication of exons 9–10. 20 test patients (no identified CNV) were selected to check for novel CNV. Data were analysed using Agilent CytoGenomics software and CNV frequencies checked against the database of genomic variants (DGV).

Results: Known CNV were detected in 16/18 patients, where array breakpoints mapped to within a minimum of ~26 bp and a maximum of ~1.3 kb of known breakpoints. In 2 patients, a novel ~4 kb 5'UTR deletion was also detected. In 2/20 test patients, a further novel 5.1 kb 5'UTR deletion was detected.

Conclusion: Custom aCGH is an effective strategy for CNV screening in *VWF*. In addition to known CNV, two novel 5'UTR deletions of ~4 kb and ~5 kb, approximately 11 kb and 23 kb upstream of *VWF* were detected. These findings could have important implications for our understanding of VWF levels in VWD and in the normal population.

Disclosure of Interest: None declared.

OR087

Long-term expression of von willebrand factor VIA sleeping beauty transposon-mediated gene therapy

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Background: Gene therapy is a promising treatment option for severe von Willebrand disease given the long-term therapeutic potential. Using a non-viral Sleeping Beauty (SB) transposon, we previously achieved long-term but insufficient levels of VWF expression in VWF-deficient mice (33% of wild-type levels 6 months after gene transfer).

Aims: To achieve long-term supraphysiological expression of VWF using a sandwich-design SB transposon specifically created to efficiently transpose larger transgenes.

Methods: Both the SB-sandwich transposon containing VWF under control of a liver-specific promoter and the transposase plasmid encoding SB100x transposase were targeted to the liver of VWF-deficient mice via hydrodynamic gene delivery. After gene transfer, long-term VWF expression and tail-clip bleeding were assessed.

Results: Use of the sandwich SB transposon dramatically increased VWF expression after gene transfer. Four months after gene delivery, VWF-plasma levels remained $310.6 \pm 101.5\%$ of wild-type levels ($n = 11$), which is ten times higher than experiments with the classic design SB transposon. As expected, bleeding phenotype was corrected 1 week after gene delivery. Surprisingly, despite high VWF levels 3 months after gene transfer, bleeding was still not totally corrected. Blood loss of treated mice was $1150 \pm 360 \times 10^6$ RBC ($n = 5$), in comparison to $205 \pm 92 \times 10^6$ RBC ($n = 28$) in wild-type mice and $1868 \pm 278 \times 10^6$ RBC ($n = 22$) in VWF-deficient mice. Interestingly, a decrease in VWF high molecular weight (HMW) multimers over time was observed, which might influence long-term phenotypic correction.

Conclusion: The powerful sandwich SB transposon system permits robust and long-term supraphysiological VWF-expression essential for VWD gene therapy. Why long-term HMW VWF multimerization is impaired in hepatocytes is currently under investigation.

Disclosure of Interest: None declared.

OR088

Safety, efficacy and pharmacokinetics of a recombinant von willebrand factor in patients with severe von willebrand disease: a prospective clinical trial

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Background: Recombinant von Willebrand factor (rVWF) could provide an alternative replacement therapy for treatment of von Willebrand disease (VWD).

Aims: Assess the safety, efficacy, and pharmacokinetics (PK) of rVWF in severe VWD, defined as type 3 (VWF:Ag ≤ 3 IU dL⁻¹), 1 (VWF:RCo < 20 IU dL⁻¹), 2A (VWF:RCo < 20 IU dL⁻¹), 2N (FVIII:C $< 10\%$), 2B or 2M.

Methods: Bleed control with rVWF:rFVIII [1.3:1] for the first infusion, and rVWF alone after a hemostatic FVIII:C level was achieved, was rated on a nominal 4-point scale (none = 4 to excellent = 1). PK was assessed in a crossover (rVWF vs. rVWF:rFVIII) and a repeated PK design. Subjects gave informed consent and the trial was approved by independent ethics committees.

Results: The treatment success rate (mean efficacy score of < 2.5) was 100% (90% CI: 87.3 to 100.0) ($n = 22$: 17 type 3, 4 type 2A, 2 type 2N; 192 bleeds: 122 minor, 61 moderate, 7 major, 2 unknown). Treatment was good (3.1%) or excellent (96.9%) in all bleeds, with excellent ratings for 5/6 gastrointestinal, 57/59 joint, 103/106 mucosal (including 31 menorrhagia, 41 epistaxis, 26 oral) and 36/37 bleeds in other locations. A single infusion of rVWF together with rFVIII for 94.8% was effective to control 81.8% of all bleeds (range: 1–4), and a median of 2 infusions (range 1–3) were required to control major bleeds. rVWF induced rapid and sustained stabilization of FVIII:C. The rVWF PK profile was unaffected by rFVIII (mean VWF:RCo terminal half-life = 21.9 h [rVWF] and 19.6 h [rVWF:rFVIII]). No inhibitors, anti-VWF binding antibodies or antibodies against host cell proteins were detected, and no severe allergic reactions or thrombotic events occurred. Two serious adverse events (AEs) (chest discomfort and increased heart rate concurrently in a subject with cryoprecipitate allergy) and 6 non-serious AEs were related to rVWF.

Conclusion: rVWF was effective and safe for bleed control in severe VWD and stabilized FVIII:C thereby eliminating the need for rFVIII after initial infusion.

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OR089

No difference in VWF levels NOR VWF survival in index cases and family members with and without P.Y1584C enrolled in zimmerman program for the molecular and clinical biology of VWD (ZPMCB-VWD)

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Background: p.Y1584C has been reported in mild VWD type 1 and is associated with reduced VWF survival and mildly increased ADAM-TS13 degradation in blood group O subjects. It was identified in 14% and 8% of Index Cases (IC) from Canadian type 1 and MCMDM-1 VWD studies.

Aims: To report the prevalence and survival effect of p.Y1584C in ZPMCB-VWD subjects.

Methods: VWF:Ag and VWFpp levels were measured and VWFpp/VWF:Ag (pp/Ag) ratio calculated as a marker of clearance. Sequencing of 636 IC and 240 Healthy Controls (HC) and targeted sequencing of affected and unaffected family members (AFM and UFM) identified p.Y1584C in 28 IC, 24 AFM, 16 UFM and 1 HC for a prevalence of 4.4% in IC.

Results: In blood group O subjects (O), there was no difference in mean ratio between p.Y1584C and type 1, but there was a significant difference ($P < 0.0001$) between p.Y1584C and type 1C (clearance) (ratio 1.7 vs. 6.0). A similar finding was observed in non-O sub-

jects. The ratio in p.Y1584C O (1.7) vs. non-O (1.4) was statistically significant ($P = 0.001$), but in the normal range (0.5–2.0). To eliminate influence of other sequence variants, 19 families with only p.Y1584C were further examined. Their phenotype was mild, regardless of the presence or absence of p.Y1584C with mean VWF:Ag (IU dL⁻¹) of 48 in IC, 45 in p.Y1584C AFM, 38 in p.Y1584 AFM, 85 in p.Y1584C UFM, and 83 in p.Y1584 UFM. Mean pp/Ag ratios in AFM with and without p.Y1584C were 1.6 and 1.7, and differences were not significant from IC ($P = 0.44$; 0.54) nor each other ($P = 0.89$). UFM with and without p.Y1584C had mean ratios of 1.2 and 1.1, and were not significantly different from each other ($P = 0.75$) but were significantly different from AFM ($P = 0.003$; 0.007).

Conclusion: p.Y1584C does not appear to have a substantial effect on VWF survival as evidenced by a pp/Ag ratio within the normal range. Low VWF:Ag levels seen in IC and AFM cannot be explained by the presence of p.Y1584C alone; additional studies are needed to determine the cause of mild type 1 phenotype in these families.

Disclosure of Interest: None declared.

OR090

Prevalence and risk factors associated with hypertension in von Willebrand disease

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Background: Increasingly, hypertension (HBP) is being recognized in individuals with congenital bleeding disorders, but little is known in those with von Willebrand disease (VWD). HBP is a risk factor for atherosclerotic heart disease (ASHD), which increases with von Willebrand factor (VWF) levels; and together with dyslipidemia and insulin resistance, contributes to metabolic syndrome. Whether VWD protects against HBP, ASHD or metabolic syndrome, is unknown.

Aims: The aim was to determine the prevalence and risk factors for HBP in VWD.

Methods: We evaluated discharge data, 2009–2011, from the Nationwide Inpatient Sample (NIS) of the Healthcare Cost and Utilization Project (HCUP), which collects a 20% sample of U.S. inpatient discharges. Discharges among adults with and without VWD were evaluated by age, race, year, severity of illness. The odds ratio for HBP was estimated by univariate and multivariate logistic regression.

Results: The prevalence of HBP in VWD patients ($n = 7556$), was 37.4%, significantly less than in non-VWD patients ($n = 19,918,970$), 49.4%, $P < 0.001$. Associated co-morbidities were also less common in VWD than non-VWD: ASHD, 21.42% vs 30.8%, hyperlipidemia, 33.8% vs 41.5%, and diabetes, 24.4% vs 36.4%, all $P < 0.001$. VWD with HBP were more likely younger, 63 vs 68 year, Caucasian, 83.5% vs. 69.8%, and female, 68.8% vs. 54.0%, than non-VWD all $P < 0.001$. Compared with VWD without HBP, VWD with HBP were older, 63 vs. 42 year, male, 31.2% vs 21.6%, with greater comorbidity: ASHD, 21.4% vs 4.7%, diabetes, 24.4% vs 7.0%, and hyperlipidemia, 33.8% vs 7.4%, all $P < 0.001$. The odds of HBP in patients with VWD was significantly less than in non-VWD, OR = 0.61 (CI 0.58–0.64), $P < 0.001$, and after adjustment for ASHD, OR = 0.91 (CI 0.89–0.92) and gender, OR = 0.34 (CI 0.34–0.35).

Conclusion: The risk of HBP is significantly lower in VWD than non-VWD patients, and associated with lower risk of ASHD, hyperlipidemia, and diabetes. Prospective studies are needed to confirm these findings.

Disclosure of Interest: None declared.

Antiphospholipid antibodies – I

OR091

Effects of antiphospholipid antibodies on vascular smooth muscle cells

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Background: Activation of platelets, monocytes and endothelial cells antiphospholipid antibodies (aPL) has been proposed as one of the pathogenic mechanisms contributing to the antiphospholipid syndrome (APS).

Aims: We determined effects of aPL on characteristic functions of human vascular smooth muscle cells (hVSMCs).

Methods: Total IgG were purified from sera of 10 patients with APS by chromatography on protein G. Thrombin generation was monitored using calibrated automated thrombography (CAT) at the surface of adherent cultured normal hVSMCs incubated with IgG (250 µg mL⁻¹) from APS patients or healthy controls, and with or without activated protein C (APC). Proliferation of hVSMCs in response to stimulation by IgG from patients or controls was studied in medium without fetal bovine serum (FBS). Negatively-charged phospholipids were quantified in the cell supernatant. hVSMC micro-particles (MPs) release was measured by flow cytometry in medium without FBS.

Results: Thrombin generation was markedly increased after incubation with IgG from all patients irrespective of the aPL concentrations in sera compared to control IgG or to medium without IgG. The increase ranged from 2-fold to 4-fold for triple positive patients (anti-β₂GPI antibodies, anticardiolipin and lupus anticoagulant). In addition, IgG from the majority of APS patients induced a resistance to APC. For all patients, procoagulant activity increase without FBS in parallel with increased apoptosis (75%) compared with unstimulated cells or cells stimulated with IgG control. IgG of all patients induced significant increase of MPs released from hVSMCs (from 200% to 300%).

Conclusion: The results show the activating effects of aPL on hVSMCs: thrombin generation increased and this hypercoagulability depends on aPL antibody profiles. The aPL-induced release of MPs and apoptotic cells induction may represent crucial mechanisms driving increased thrombin generation.

Disclosure of Interest: None declared.

OR092

Impact of hydroxychloroquine treatment on pregnancy outcome in patients with antiphospholipid antibodies

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Background: Obstetric morbidity in antiphospholipid syndrome (APS) includes recurrent first trimester loss, stillbirth, intrauterine death, pre-eclampsia, premature birth and fetal growth restriction. Low dose aspirin and low molecular weight heparin have improved pregnancy outcome, but reduction in pregnancy loss remains sub-optimal. The antimalarial hydroxychloroquine (HCQ) has been used for decades in patients with SLE.

Aims: To assess the pregnancy outcome in women with aPL treated with HCQ during pregnancy.

Methods: This observational, retrospective, single centre clinical study included 170 pregnancies in 96 women with persistent aPL. Sixty-five consecutive pregnancies occurred in 31 women treated with HCQ for at least 6 months prior to pregnancy. HCQ was continued throughout gestation (group A). One-hundred nineteen consecutive pregnancies

occurred in 65 women with aPL who had not been treated with HCQ prior to conception acted as controls (group B).

Results: HCQ treatment was associated with a significantly higher rate of live births (66.7% in group A vs. 57.1% in group B, $P = 0.05$) and a lower rate of pregnancy morbidity (47.1% in group A vs. 63.0% in group B, $P = 0.004$). We observed a higher prevalence of spontaneous vaginal labour in HCQ-treated women compared to group B (37.3% vs. 14.3%, $P = 0.01$). Pregnancy duration was longer in group A than B (27.6 [6–40] weeks vs. 21.5 [6–40], $P = 0.03$). Fetal losses beyond 10 weeks gestation were less frequent in group A than B (2% vs. 10.9%, $P = 0.05$). Placenta mediated complications (pre-eclampsia, abruption placenta and intrauterine growth restriction (IUGR)) were less prevalent in HCQ treated women compared to controls (2% vs. 10.9%, $P = 0.05$). The association of HCQ with the absence of any complications in pregnancy was confirmed after multivariate analysis (OR 2.2; 95%CI[1.2–136.1]; $P = 0.04$).

Conclusion: Women with aPL may benefit of treatment with HCQ during pregnancy. The addition of HCQ to conventional treatment is worthy of further assessment.

Disclosure of Interest: None declared.

OR093

Thrombin generation in patients with antiphospholipids antibodies

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Background: Scarce information is available on thrombin generation in patients with antiphospholipid antibodies (aPL). A calibrated automated thrombogram (CAT) assay modified to simulate the clotting status within the microcirculation was developed and validated (Thromb Res 2014;134(Suppl2):S97).

Aims: To evaluate the sensitivity of the modified CAT according to aPL reactivity in patients with aPL.

Methods: Platelet poor plasma samples from 112 anticoagulation-free patients, (28 M/84 W, mean age 57.4 ± 16.4 yrs) with at least one abnormal test results for aPL (aCL IgG/M, a β_2 -GPI IgG/M, lupus anticoagulant, LA), and from 105 apparently healthy controls (HC, 50 M/55 W, mean age 44.0 ± 9.3 yrs) were tested in a modified CAT using an intermediate tissue factor concentration (3 pM) in the presence of 5 nM thrombomodulin and 116 nmol fondaparinux. Lag time (LT), peak velocity (PK), acceleration (peak/(time to peak-lag time), ACC), true tail (starttail-time to peak, TT), and endogenous thrombin potential (ETP) were normalized according to the results obtained in normal platelet poor plasma, with ratios > 1.0 indicative of hypercoagulability.

Results: In the overall aPL population, compared to HC, significant differences were observed for LT (0.57 ± 0.20 vs 0.95 ± 0.17 , $P < 0.0001$), PK (1.41 ± 0.98 vs 1.07 ± 0.55 , $P = 0.02$), and ACC ratios (1.69 ± 1.56 vs 1.05 ± 0.58 , $P = 0.001$). In LA+ patients ($n = 77$), the LT ratio was the only abnormal parameter (0.45 ± 0.24 , $P < 0.0001$), irrespective of the association with either aCL-reactivity ($n = 2$), a β_2 -GPI-reactivity ($n = 8$) or both ($n = 27$; $P \geq 0.13$). LT was also prolonged in the 35 LA- patients (0.83 ± 0.19 , $P < 0.01$), especially in patients with double reactivity (0.75 ± 0.18 , $P = 0.04$). ACC and PK ratios were increased only in LA- patients ($P < 0.0001$), irrespective of aPL reactivity ($P \geq 0.56$).

Conclusion: In the modified CAT assay, an isolated, marked prolongation of the LT parameter is the hallmark of lupus anticoagulants.

Disclosure of Interest: None declared.

OR094

Inflammation and cardiovascular risk factors are associated with recurrent thrombosis in antiphospholipid syndrome

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Background: Antiphospholipid syndrome (APS) is a pro-thrombotic autoimmune disease that affects different vascular beds, with potential risk for recurrence. Yet, factors associated with thrombosis recurrence are not completely elucidated.

Aims: To determine possible demographic, clinical and laboratory markers associated with thrombosis recurrence in APS

Methods: Retrospective evaluation of a cohort of APS patients with previous thrombosis, by medical records and specific questionnaires

Results: One hundred patients were included, 63% had primary APS. The first thrombotic event was venous (VT) in 69% and arterial (AT) in 31%. History of recurrent thrombosis was positive in 41% of patients, the mean number of thrombotic episodes was 2.5 (2–4) per patient and ¼ of them were on oral anticoagulants at the time of recurrence. Recurrence rate was similar between patients with AT and VT (53% and 40%, respectively, $P = 0.2$). In more than 80% of cases, the further thrombotic episodes occurred in the same type of vessel (arterial or venous) of the first episode. Age, gender, diagnosis of lupus, antiphospholipid antibodies profile were not associated with recurrence. Among AT patients, higher levels of LDL cholesterol (> 107 mg dL⁻¹) were associated with recurrent events (OR = 15, 95% CI = 1.1–218.3, $P = 0.04$, ROC area = 0.813). Among VT patients, the conditions associated with recurrence were: hypertension (OR = 4.5, 95% CI = 1.1–17.6; $P = 0.03$), higher neutrophils (OR 4.4, 95% CI = 1.4–13.3, $P = 0.008$) and monocytes counts (OR 4.5, 95% CI = 1.4–13.9, $P = 0.009$) and higher serum levels of complement C4 (OR 3.3, 95% CI = 0.98–11.1, $P = 0.05$).

Conclusion: The results suggest that, in APS, factors associated with thrombosis recurrence differ according to the site of the first thrombosis. Comorbidities that confer cardiovascular risk may be associated with AT and VT recurrence. However, VT recurrence was also associated with inflammatory response, since neutrophils and monocytes counts, as well as C4 levels, were higher in patients with multiple thrombosis

Disclosure of Interest: None declared.

OR095

Obstetric antiphospholipid syndrome: high comparative prevalence of common mental disorders during follow-up

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Background: Neurological manifestations are described in the thrombotic antiphospholipid syndrome (APS), but the occurrence of psychi-

atric disorders is uncertain in obstetric APS (oAPS) and there is no comparative study on women sharing initial clinical manifestations.

Aims: To study the comparative prevalence of common mental disorders in oAPS; to look for an association with the antiphospholipid antibody (aPLAb) subtypes.

Methods: The NOH-APS cohort (Blood 2012; 119: 2624–32) recruited women with no vascular antecedent but significant unexplained obstetrical wastage (3 abortions or one fetal death), categorized according to the results of laboratory screening: oAPS ($n = 517$), thrombophilia ($F5 rs6025$ or $F2 rs1799963$: $n = 279$) and controls (negative screening; $n = 796$). We assessed the prevalence of common mental disorders using the diagnostic psychiatric questionnaire MINI 5.0.0 (Sheehan DV, Lecrubier Y, J Clin Psychiatry 1998) 10 years after inclusions. The prevalence of disorders was compared between groups and adjusted risk models were computed.

Results: 1539 patients could be evaluated. No difference was evidenced between thrombophilic and control women. The oAPS women had more mood disorders (recent depressive episodes, recurrent depressive episodes), more anxiety disorders (general anxiety, panic disorders, agoraphobia, social phobia, post-traumatic stress) but no more psychotic disorders. Lupus anticoagulant (LA) and anti-b2-Gp1 IgG (ab2Gp1-G) increased the risk of mood disorders detection while anti-cardiolipin IgG (aCL-G) was protector. LA and aCL-G increased the risk of anxiety disorders detection. ACL-G was associated with the presence of psychotic disorders.

Conclusion: Women with oAPS are at increased risk of psychiatric symptoms during follow-up. Early specific medical coverage is needed and studying the effects of aPLAbs on brain biology is warranted.

Disclosure of Interest: None declared.

Vitamin K antagonists

OR096

Quality of vitamin k antagonist control and 1-year outcomes: a global perspective from the garfield-af registry

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Background: Optimal international normalized ratio (INR) levels are hard to achieve in patients on vitamin K antagonists (VKA). Time in therapeutic range (TTR) estimated by Rosendaal method is an indicator of quality of VKA control.

Aims: To study TTR of INR in patients with newly diagnosed non-valvular atrial fibrillation in relation to demographics, care settings and 1-year outcomes, using the target INR range of 2.0–3.0.

Methods: GARFIELD-AF enrolled 17,168 prospective patients. 8445 received VKA. TTR was estimated for patients with ≥ 3 INR values during 1-year follow-up. TTR $> 60\%$ and $> 70\%$ were explored as indicators of acceptable or good control. Hazard ratios (HRs) for outcomes were adjusted for CHA₂DS₂-VASc. Informed consent from patients and ethics committee approval were obtained.

Results: 4967 patients with TTR were analyzed. The median number of INR/patient was 12 (interquartile range 7–19). 49.6% and 29.0% of patients had TTR $> 60\%$ and $> 70\%$, respectively. Using a 60% TTR cut-off ($\leq 60\%$ vs $> 60\%$), heavy alcohol consumption was more frequent in patients with poor control (3.1% vs 1.5%). Fewer patients diagnosed at a hospital had TTR $> 60\%$ (46.2%) vs patients diagnosed at an outpatient office (57.3%) or anticoagulation clinic/thrombosis center (57.1%). Patients with poor control had higher risk of death (HR 2.87, 95% confidence interval, 1.97–4.19) and stroke/systemic embolism (SE) (1.98, 1.13–3.47) than those with TTR $> 60\%$. The equivalent HR for major bleeding was 1.31 (0.78–2.20). All results were similar with a 70% cut-off. The HR for stroke/SE increased from 1.98 (1.13–3.47) to 2.83 (1.38–5.78) when the TTR cut-off was increased from 60 to 70%. We also analyzed 3478 patients with missing TTR, who had a similar mortality risk to those with TTR $\leq 60\%$, but lower risk of stroke/SE and major bleeding; these results need further investigation.

Conclusion: These data show that poor VKA control is associated with increased risk of mortality and stroke/SE. Also, increasing TTR from 60 to 70% may reduce the risk of stroke/SE.

Disclosure of Interest: H. ten Cate Grant/Research Support from: Bayer, Boehringer Ingelheim, AstraZeneca, Consultant for: Philips, Speaker Bureau of: Bayer, Boehringer Ingelheim, GSK, Leo, Roche, Stago, S. Haas Consultant for: Bayer, Bristol-Myers Squibb, Daiichi Sankyo, Pfizer, Sanofi, Speaker Bureau of: Bayer, Bristol-Myers Squibb, G. Accetta: None Declared, P. Angchaisuksiri: None Declared, J. Camm Consultant for: Bayer, Biotronik, Boehringer Ingelheim, Boston Scientific, Bristol Myers Squibb, Cardiovascular Therapeutics, ChanRx, Daiichi, Medtronic, Menarini, Mitsubishi, Novartis, Richmond Pharmacology, Sanofi Aventis, Servier, St Jude Medical, Takeda, Xention, Speaker Bureau of: Pfizer, R. Corbalan: None Declared, J. Eikelboom Consultant for: AstraZeneca, Bayer HealthCare, Boehringer Ingelheim, Bristol-Myers Squibb, Corgenix, Daiichi Sankyo, Eisai, Eli Lilly, GlaxoSmithKline, Haemoscope, Johnson & Johnson, McNeil, Pfizer, Portola and Sanofi-Aventis, D. Fitzmaurice Consultant for: Bayer, Boehringer Ingelheim, Sanofi Aventis, Bristol-Myers Squibb, Daiichi, Janssen, Portola, S. Goldhaber Grant/Research Support from: Bristol-Myers Squibb, BTG, Daiichi, NHLBI, Consultant for: Boehringer-Ingelheim, Bristol-Myers Squibb, Daiichi, Janssen, Portola, S. Goto Grant/Research Support from: Sanofi Aventis, Pfizer, Consultant for: Bristol-Myers Squibb, Bristol-Myers Squibb/Pfizer, Bayer, Daiichi Sankyo, Sanofi Aventis, Medtronic, Speaker Bureau of: Bristol-Myers Squibb/Pfizer, B. Jacobson Consultant for: Bayer HealthCare, Sanofi Aventis, G. Kayani: None Declared, A. Turpie Consultant for: Janssen, Bayer Healthcare, Boehringer Ingelheim, Pfizer, Bristol-Myers Squibb, Speaker Bureau of: Janssen, Bayer Healthcare, Boehringer Ingelheim, Pfizer, Bristol-Myers Squibb, A. Kakkar Grant/Research Support from: Bayer Healthcare, Boehringer-Ingelheim Pharma, Bristol-Myers-Squibb Co, Eisai Inc, Pfizer Inc, Sanofi SA, Consultant for: Bayer Healthcare, Boehringer-Ingelheim Pharma, Bristol-Myers-Squibb Co, Daiichi Sankyo Europe, Eisai Inc, Pfizer Inc, Sanofi SA.

OR097

Independent predictors of poor vitamin k antagonist control in venous thromboembolism patients: data from the einstein-dvt and pe studies

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Background: Vitamin K antagonists (VKA) are used to prevent recurrent disease in patients with venous thromboembolism (VTE). Their

efficacy and safety depend on individual time in therapeutic range (iTTR) and variability of International Normalised Ratios.

Aims: We aimed to identify independent predictors of poor VKA control > 28 days.

Methods: In a prospective cohort of 3825 VTE patients, separate logistic regression analyses were performed to identify predictors of low iTTR ($\leq$ lower quartile) and instability (iTTR < median and variability > median). Subsequently, the correlation between these predictors and clinical outcome was investigated in Cox models. The EINSTEIN studies were approved by the institutional review board of each centre, and written informed consent was obtained from all patients.

Results: Fragility (odds ratio [OR] = 1.53), age > 75 years (OR = 0.58), malignancy (OR = 1.48), secondary VTE (OR = 1.42), and INR < 2.0 at stop of double therapy (OR = 1.35) were independent predictors of low iTTR. Only the first three were also predictive for instability (OR = 1.50, OR = 0.59 and OR = 1.90, respectively). Predictive values of early ($\leq$ 28 days) low iTTR and instability depended on type of VKA. In acenocoumarol users, only early low iTTR was an independent predictor of subsequent low iTTR (OR = 1.93) and instability (OR = 1.54). In warfarin users, early low iTTR (OR = 1.35) and instability (OR = 1.25) were additionally predictive for low iTTR, but only the latter was predictive for instability (OR = 1.92). Many predictors of VKA control predicted also premature discontinuation, but few were prognostic for thrombotic or bleeding events.

Conclusion: We identified several independent predictors of low iTTR and instability, which showed some similarities but did not fully overlap. Early VKA control was of additional value for prediction of both, but had to be interpreted in the context of VKA type.

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OR098

Bleeding risks are high in elderly persons who use vitamin k antagonists combined with platelet aggregation inhibitors

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Background: Patients with atrial fibrillation (AF) frequently require vitamin K antagonists (VKAs) and at times additional platelet aggregation inhibitors. Combining platelet aggregation inhibitors and VKAs increases the risk of major bleeds two- to fourfold. This risk combined with the age-related increase in baseline bleeding risk may be a contraindication for combination therapy in elderly. However, data are lacking on absolute bleeding risks stratified by age.

Aims: To examine the bleeding risk for VKAs and/or platelet aggregation inhibitors in AF patients stratified by age.

Methods: This nationwide cohort study focused on Danish patients aged 50 years or older diagnosed with incident AF between 1995 and 2013. Seven exposure categories (based on a prescription database data) were considered: VKAs, aspirin, clopidogrel, dipyridamol, a combination of two platelet aggregation inhibitors, a platelet aggregation inhibitor combined with a VKA, and triple therapy. Incidence rates (IRs) of major bleeds per 100 patient-years and 95% confidence intervals (CIs) were stratified by 10-year age categories.

Results: 207,085 (87%) of 238,555 AF patients filled at least one prescription for an anticoagulant. 35,399 major bleeds occurred during a

follow-up of 1,015,511 patient-years. For monotherapy, the overall IR for bleeds was 1.78 (95%CI 1.64–1.92) among patients aged 50–60 years. IRs increased to 4.79 (95%CI 4.29–5.34) and 4.45 (95%CI 4.20–4.72) for patients aged over 90 years taking VKAs and aspirin respectively. Overall IRs during triple therapy were three to five times higher (IR range: 9.02 to 20.27) compared with VKA monotherapy. IRs for triple therapy were 14.18 (95%CI 11.94–16.72) and 20.27 (95%CI 11.78–32.68) for patients aged above 80 and 90 years, respectively.

Conclusion: Major bleeding rates are high in AF patients aged 80 years or older who receive triple therapy. To judge whether triple therapy is contraindicated in particular age groups, data are needed on its preventive effect on ischemic stroke.

Disclosure of Interest: None declared.

OR099

The effect of CYP2C9 polymorphism on inr decline in patients stopping warfarin before surgery

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Background: Patients on warfarin scheduled for elective surgery are normally required to stop treatment for 5 days prior to the procedure taking place in order to minimise the risk of per-operative bleeding. However, the anticoagulant activity of warfarin subsides at different rates among different patients which can lead to postponements or cancellations of surgery. The variable rate of fall in INR could be explained by variability in warfarin clearance influenced by patient genetics.

Aims: To investigate the potential influence of CYP2C9 polymorphism on the rate of fall in INR in patients withdrawing from warfarin prior to elective surgery.

Methods: 152 patients on chronic warfarin therapy (aged 43–93 years) who were scheduled for elective surgery were recruited. A single blood sample was taken for CYP2C9 genotyping after obtaining informed consent. INR was measured on days 1 and 5 of warfarin withdrawal. Information about patients' comorbidities, concurrent use of drugs, weight and height were recorded.

Results: For patients with two CYP2C9 variant alleles (*2*2 or *2*3), the odds of having an INR $\geq$ 1.5 before the planned day of surgery were 8.64 times greater (95% CI = 2.25, 33.25) than for other patients. Multiple regression analysis revealed that the rate of INR decline is reduced in the presence of two CYP2C9 variant alleles as well as increasing patient age, weight and number of comorbidities and is increased with increasing initial INR [F(5, 132) = 242.9, $P < 0.0001$], all of which accounted for approximately 90% of the inter-individual variability in INR decline.

Conclusion: A genotype-guided protocol to tailor warfarin withdrawal according to individual patient's CYP2C9 genotype could reduce costs through a reduction in cancellation or delays of planned procedures and could also be beneficial when transitioning patients from warfarin to one of the new oral anticoagulants.

Disclosure of Interest: None declared.

OR100

Impact of vitamin k antagonists (VKA) on quality of life (QoL) in a prospective cohort of 807 atrial fibrillation (AF) patients

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Background: VKA reduce the risk of ischemic stroke in AF patients, but many patients are reluctant to take it. One of the reasons is the expected burden of complex dosing, blood monitoring and food and drug interactions. However, it is unclear to what extent VKA use influences QoL.

Aims: Analyzing the impact of VKA initiation on QoL, and the influence of changes in patient and treatment characteristics on VKA perception.

Methods: In a prospective cohort of 240 new and 567 long-term VKA users, generic QoL and VKA perception (satisfaction and convenience) were measured at inclusion and at 3 months by the validated SF-36 and PACT-Q questionnaires (scores range 0–100). Effect sizes [ES] were determined according to Cohen (small = 0.20, medium = 0.50). Data on age, sex, comorbidity, comedication, time in therapeutic range (TTR), monitoring frequency, no. of tablets, bleeding and thromboses were collected. According to Dutch law, no approval by the institutional review board was needed. All patients gave written informed consent.

Results: In new patients, treatment convenience (median: 95) increased with age, and was lower after bleeding in patients <65 years (regression coefficient [RC] -12). Satisfaction (median: 64) was not related to any factor. Six out of 8 SF-36 domains improved after 3 months (ES 0.27–0.37). Then, scores were comparable to the long-term patients and the general Dutch population. The latter had only slightly higher scores for physical role (ES 0.19) and general health (ES 0.17). In long-term patients, increase in convenience was associated with improved TTR (RC 0.03; r^2 0.01). Satisfaction decreased in case of more comedication (RC -7.0; r^2 0.02).

Conclusion: The initially lower QoL improved 3 months after VKA initiation to a level that was comparable to long-term patients and even the general population. Convenience was overall high, but lower in younger patients. Satisfaction was moderate. Changes in patient and treatment characteristics were either not or only weakly related to VKA perception.

Disclosure of Interest: None declared.

Hemophilia – prophylaxis

OR101

Spinart trial 3-year results with bayer's sucrose-formulated recombinant factor VIII: relationship between bleeding frequency and joint health in adults with severe hemophilia A using prophylaxis

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Background: The 3-year, open-label, randomized, controlled, parallel-group SPINART study compared prophylaxis vs on-demand treatment with Bayer's sucrose-formulated recombinant factor VIII (rFVIII-FS) in adults and adolescents with severe hemophilia A.

Aims: To examine the relationship between bleeding frequency and joint health at year 3 of SPINART

Methods: SPINART included males aged 12–50 years with severe hemophilia A, ≥ 150 exposure days to FVIII, 6–24 bleeding events or treatments in the previous 6 months, and without an inhibitor or prophylaxis for > 12 months during the past 5 years. Patients received rFVIII-FS either on demand for treatment of bleeding events or 25 IU kg⁻¹ 3 times/wk as prophylaxis. Study outcomes included bleeding frequency (primary) and changes in the Colorado Adult Joint Assessment Scale (CAJAS; secondary endpoint), with increases from baseline indicating worse joint health. Data for the intent-to-treat population ($n = 84$, 42 per group) are presented.

Results: Change from baseline to year 3 in CAJAS total score was 0.63 for on demand and -0.31 for prophylaxis (least squares [LS] mean difference, -0.94; 95% CI, -1.61 to -0.26; $P = 0.0072$ by constrained longitudinal data analysis). LS mean change from baseline at years 1 and 2 was 0.19 and 0.34 for on demand and -0.46 and -0.57 for prophylaxis, respectively. For patients on prophylaxis with no bleeds ($n = 14$), 1–10 bleeds ($n = 18$), 11–50 bleeds ($n = 6$), and > 50 bleeds ($n = 1$) during the study, mean change from baseline CAJAS total score at year 3 was -0.89, -0.37, -0.08, and 1.17, respectively, using last observation carried forward (postbaseline CAJAS scores were not available for 3 patients on prophylaxis).

Conclusion: Patients with severe hemophilia A who received secondary prophylaxis with rFVIII-FS during SPINART experienced significant reduction in bleeding frequency and improvement in joint health as measured by CAJAS at 3 years compared with patients treated on demand.

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OR102

Discontinuing early prophylaxis in young adults with severe haemophilia A: deterioration of joint status after 10 years despite low bleeding rates

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Background: Although the efficacy of early prophylaxis in haemophilia is well established, up to 35% of patients switch to on demand treatment in adulthood while maintaining a low bleeding frequency and similar joint status. However, so far follow-up after stopping was limited (≤ 4.6 years).

Aims: This study assesses the long-term consequences of discontinuing prophylaxis started in early childhood.

Methods: Single centre cohort study in patients with severe haemophilia A, born 1970–1988, without inhibitors. Self-initiated changes in prophylaxis were recorded from starting self-infusion to last evaluation. All switches to on demand treatment for a minimum of two consecutive weeks were considered. Subjective outcome was measured by self-reported joint bleeds in the last 3 years and the Haemophilia Activities List (HAL, range 100–0). Objective outcome of the elbows, knees and ankles was measured by the Haemophilia Joint Health Score (HJHS version 2.1, range 0–124 points) and the radiological Pet-

tersson score (range 0–78 points). Differences in outcome parameters were analysed using log-linear regression adjusted for age.

Results: Out of 66 patients, evaluated at a median age of 32.4 years and follow up of 16.6 years, 27 (41%) patients discontinued prophylaxis at least once: 10 interrupted and 17 patients stopped prophylaxis permanently. Self-reported and objective outcome for patients who stopped prophylaxis were compared to outcome in patients who continued prophylaxis. At a median of 10.1 years (IQR:5.9–13.8) after stopping prophylaxis, patients who stopped showed worse arthropathy (HJHS: 23 vs. 14; Pettersson: 16 vs. 5; $P < 0.01$), while self-reported outcome was similar in both groups (1.2 joint bleeds/year; HAL: 84).

Conclusion: In spite of good self-reported outcome, significant deterioration of joint status was seen ten years after stopping early prophylaxis. These results support the need for continued prophylaxis in adults with severe haemophilia and careful monitoring of joint status using objective tools.

Disclosure of Interest: None declared.

OR103

Retrospective 28-year long term follow-up of prophylaxis in a cohort of 49 patients with severe haemophilia A

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Background: Prophylaxis in children with severe haemophilia A represents the gold standard treatment regimen to prevent bleeding and subsequent joint disease. This treatment regimen became the therapy standard at the Bonn haemophilia Center at the mid 70ies.

Aims: Long-term evaluation of an intensive prophylaxis regimen in children with severe haemophilia A.

Methods: Retrospective analysis of 49 patients with severe haemophilia A who started a prophylactic regimen in 1978 between the age of 1–16 years were evaluated in 2006. Petterson scores were available from X-rays from ankle, knee and elbow joints taken every 3–5 years. Gilbert scores were assessed annually. Concentrate consumption as well as number of total bleeds and joint bleeds were taken from the patients' records.

Results: After 28 years only 13% of the patients had normal Petterson scores for an ankle joint, 42% for a knee joint and 53% for an elbow joint. The ankle joint was the first to develop pathological Petterson scores (defined as score > 1) after a median time of 10 years, followed by the knee joint and elbow joints with median times of 20 and 25 years, respectively. The Gilbert scores became pathologic about one decade after the Petterson scores. The number of total bleeds were 6.1/year and 0.2/year for joint bleeds.

Conclusion: Current prophylaxis regimens can not prevent joint disease in a life time perspective, Ankle joints are the first joints developing arthropathy in patients on a prophylactic regimen. Therefore ankle joints may serve as an indicator of early joint disease and worthwhile to follow by MRI between age 7–10 years.

Disclosure of Interest: None declared.

OR104

Prophylaxis in children with severe and moderate haemophilia: a survey of UK practice

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Background: Prophylaxis reduces the risk of joint damage in severe haemophilia A (HA) and is recommended for all severely affected children. Uncertainty persists regarding optimal regimens and there are few data on its use in non-severe haemophilia.

Aims: To assess prophylaxis in severe and moderate haemophilia in UK children born between 1995–2013 and to compare this to published guidelines.

Methods: Questionnaire survey of UK paediatric haemophilia centres.

Results: 32 centres replied including 22 Comprehensive Care Centres (CCCs). 1009 children were identified, 82% HA (84% severe), 18% haemophilia B (HB) (66% severe). Overall 86% severe HA and 76% HB children were on prophylaxis, increasing to 96% and 80% respectively in those age > 3 years. Overall 2% were not on prophylaxis due to inhibitors. Prophylaxis in moderate haemophilia was 22% in HA and 9.6% in HB. 12 (55%) of CCCs use pharmacokinetic (PK) dosing (trough > 1 iu dL⁻¹) while 10 (45%) dose adjust on clinical criteria. 10 CCCs provided detailed information. From 1995–2010 age and exposure days at prophylaxis onset reduced for both HA and HB. 135/242 (56%) and 22/43 (51%) of children with HA and HB respectively commenced prophylaxis for haemarthrosis +/- soft tissue bleeding. In HA 36% started prior to a first joint bleed and 78% had ≤ 2 joint bleeds, compared with 41% and 77% in HB. 6% and 2% of cases started for ICH. The initial prophylaxis regimen in HA varied from x1 per week to alternate days. In HA 60% of children are currently on alternate day prophylaxis, while 20% are on x3 per week. Median dose is 30 iu kg⁻¹ (range 10–96). In HB 77% receive prophylaxis x2 per week with a median dose of 45 iu kg⁻¹ (range 18–69). 192/338 (57%) non-inhibitor cases used a portacath at some stage. In HA a previous inhibitor and PK dosing are associated with higher FVIII use.

Conclusion: In keeping with current guidelines the majority of children in the UK with severe haemophilia receive prophylaxis. There is however evidence of variation in how this is prescribed and monitored.

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OR105

Prophylaxis in adult patients with severe hemophilia A or B without inhibitors

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Background: Long-term prophylaxis is recommended as standard of care in children with severe hemophilia A (HA) and B (HB) without inhibitors. However, long-term prophylaxis is used less frequently in adults with severe hemophilia.

Aims: To understand the use of long-term prophylaxis (and prophylaxis regimens) in adults with severe hemophilia.

Methods: We surveyed (as of Dec 2013) hemophilia treatment centers (HTCs) in countries where long-term prophylaxis in children is routinely used.

Results: 112 HTC from 14 countries in North America, Australasia, and Western Europe reported on 3497 severe HA and 648 severe HB adults. A significantly higher proportion of HA patients were on prophylaxis (HA: 60% vs HB: 50%; $X^2 P < .001$). This was particularly true in the age group of 18–30 y [73% (HA) vs 60% (HB)]. For HA patients the use of prophylaxis was progressively less in older age groups: 31–40 y (55%), 41–50 y (46%), 51–70 y (38%) and > 70 y (27%). Surprisingly there was little drop in the proportion of HB patients on prophylaxis with increasing age (between 39 and 49% of patients in all age groups were on prophylaxis).

Most HA patients on prophylaxis (61%) were receiving ≥ 3 infusions/wk while most HB patients on prophylaxis (76%) were receiving ≤ 2 infusions/wk. Once/wk prophylaxis was reported in 3% of HA patients on prophylaxis and by 19% of HB patients on prophylaxis while daily prophylaxis was rarely reported in both groups: 5% (HA) and 2% (HB).

Conclusion: This survey captured information on 4145 adults with severe HA/HB and represents current prophylaxis use in adults with hemophilia in Western countries. Given the well documented benefits of prophylaxis in children it is reassuring to observe that HTCs are extrapolating from their experience in children to adults and that prophylaxis in adults is more prevalent than previously reported.

Disclosure of Interest: None declared.

Factor VIII – I

OR106

Transgene expression under the control of FVIII promoter for hemophilia A targeted therapy

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Background: Liver is considered the main organ producing FVIII, although other cell sources were identified and little is still known about FVIII promoter regulation. FVIII promoter (pF8) was initially described in 1995 but a fine characterization is still required.

Aims: To evaluate pF8 activity in cells and tissues and to assess if this promoter is suitable for FVIII in gene therapy application.

Methods: In silico analysis to predict transcriptional factor (TF) consensus sequences on pF8 was performed by the software PROMO 3.0. Short and Long FVIII promoter (SpF8: 1175 and LpF8: 2350) sequences were amplified by PCR and cloned in LV carrying GFP or FVIII as transgene. GFP distribution was analyzed by FACS and

immunofluorescence in cells and tissues. FVIII activity was measured by aPTT.

Results: In silico analysis of TF consensus sequences predicted the presence of several myeloid-specific TF, in addition to hepatocytes- and endothelial-specific TF. *In vitro* LV-transduction showed GFP expression mainly in several myeloid and endothelial cells and a feeble expression in hepatic cell lines. Since no differences in GFP expression between long and short pF8, we used the SpF8 for further studies. After LV injection in mice GFP expression was restricted to hepatic non-parenchymal cells, meanwhile hepatocytes were barely detected (< 1%). In particular, by costaining of GFP and liver sinusoidal endothelial (LSEC) or Kupffer cell-specific markers revealed pF8 was predominantly active in LSEC. Instead, in hematopoietic organs, such as spleen and bone marrow, GFP expression was virtually restricted to myeloid cells. We injected LV.SpF8.hFVIII in hemophilia A mice. So far, aPTT assay demonstrated FVIII activity in therapeutic range without antibody formation.

Conclusion: Our results demonstrate that pF8 is differentially active in cell-subpopulations of several organs contributing to identify the FVIII producing cells and targeting FVIII expression in these cells by LV produce FVIII in therapeutic range.

Disclosure of Interest: None declared.

OR107

Role of the IGG backbone in B cell depletion and inhibitor reversal in hemophilia A WITH anti-CD20 antibodies

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Background: Inhibitor formation against factor VIII (FVIII) is a major complication in the treatment of hemophilia A (HA). Current immune tolerance induction (ITI) regimens are not universally effective. Rituximab, a B cell-depleting antibody against CD20, has shown mixed results for inhibitor reversal in patients. Few pre-clinical data are available.

Aims: 1) To extensively characterize the depletion and recovery of B cells in multiple immune compartments with IgG2a or IgG1 antibodies against murine CD20. 2) To assess the ability of these antibodies to reverse pre-existing inhibitors in HA mice.

Methods: Inhibitors (ave. ~70 BU) were generated by weekly IV injections of FVIII in BALB/c-HA mice ($n = 7$ /group). Subsequently, anti-mCD20 (18B12) with IgG2a or IgG1 backbone was injected IV (250 μ g) at day 0 and 21 ($n = 5$ /group). One day and 2 months later, spleen, lymph nodes, bone marrow, and peripheral blood were harvested and the frequency and absolute number of B cell subsets were assessed by flow cytometry.

Results: Overall, B cell depletion was more complete using IgG2a anti-mCD20. Particularly, minimal depletion of B-10 cells (which produce IL-10), marginal zone and B-1b cells (innate-like B cells involved in T-independent humoral immunity) was observed with IgG1 anti-mCD20. Plasma cells were largely unaffected by either treatment. Recovery was mostly complete after 2 months. Both antibodies reduced inhibitor titers to ~33 BU during maximum B cell depletion. However, inhibitors eventually rebounded over a 2-month period, albeit delayed by 1 month when using IgG2a anti-mCD20 (the murine equivalent to Rituximab).

Conclusion: Although IgG1 anti-mCD20 spared potentially tolerogenic B cell subsets, IgG2a directed longer-lasting hyporesponsiveness. This reduction is promising in that it may facilitate inhibitor reversal in combination with other tolerogenic therapies. In order to also target the T cell compartment, we are currently testing this therapy in combination with other drugs.

Disclosure of Interest: G. Rogers: None Declared, A. Sherman: None Declared, D. Markusic: None Declared, H. Jiang Shareholder of: Biogen Idec, Employee of: Biogen Idec, R. Herzog Grant/Research Support from: Biogen Idec.

OR108

High throughput amino acid sequence epitope mapping of inhibitory antibodies in severe hemophilia A

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Background: Inhibitory antibodies complicate a third of severe hemophilia A (HA) cases. A high throughput epitope mapping technology could characterise inhibitors in patient cohorts to gain insight into their inhibitory mechanism.

Aims: To epitope map inhibitor samples from patients with severe HA using a novel, high throughput, ELISA based, reusable peptide microarray (Pepscan[®]).

Methods: Samples from 13 severe HA patients with known inhibitors were analysed. The mapping platform consists of parallel peptide microarrays with overlapping 20 (linear) or 15-mer (looped) libraries, providing an *in-vitro* mimic of primary and secondary structure. The sequence coverage is 98.2% (1–2290) with a cut-off defined by monoclonal antibody data.

Results: The median inhibitor titre was 26.0 BU mL⁻¹ (range 0.8–750). There were 77 regions of positive binding (linear and looped), with a median of 6 per patient (range 3–9). Binding was seen in the A1 (*n* = 12), a1 (*n* = 11), A2 (*n* = 7), a2 (*n* = 6), B (*n* = 10), a3 (*n* = 4), A3 (*n* = 7) and C2 (*n* = 1) domains (patients). 17 epitopes recurred in ≥ 2 patients. All of the epitopes represented within the FVIII crystal structure (2R7E) demonstrated solvent accessible residues for antibody interaction. 11/13 patients demonstrated binding within an A1/a1 region (316–365), containing the Factor X interaction site (337–372), a sulphated tyrosine residue (Tyr346) important for FVIII activation and the Activated Protein C inactivation site (Arg336). Binding was also seen in regions associated with von Willebrand Factor (1646–1670), activated Factor IX (701–730) interaction and sites of structural importance: disulphide bond formation (231–255, 316–365); calcium ion (96–125); zinc ion (231–255 and 316–365) and a sulphated tyrosine residue (701–730).

Conclusion: A high throughput peptide microarray offers a novel, low sample volume approach for epitope mapping in clinical studies. The sequence epitopes described present putative mechanisms of action for inhibitors to FVIII.

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OR109

Adoptive transfer of *in vitro* expanded FVIII-specific regulatory T cells to mediate tolerance in hemophilia A mice

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Background: The current standard of care for Hemophilia A (HemA) is protein replacement therapy, but complications arise in one-third of patients from the development of inhibitory antibodies to factor VIII (FVIII). The immune response also hinders the long-term potential for gene therapy treatments.

Aims: We have pursued development of a protocol to expand regulatory T (Treg) cells specific to FVIII for modulating anti-FVIII immune responses. Antigen-specificity could reduce the risk of off-target suppression without compromising long-term tolerance.

Methods: Treg cells are isolated from FVIII-sensitized mice, sorted on CD25^{high} marking, and subsequently expanded specifically with FVIII, APCs and IL-2. Afterwards, Treg cells are further expanded with anti-CD3/anti-CD28 beads, anti-Crry, and IL-2. Adoptive transfer experiments into HemA mice are performed to test *in vivo* suppressive function of expanded Treg cells.

Results: After a 10–20 fold overall expansion of activated Treg cells, *in vitro* FVIII-specific suppressive assays of non-specifically expanded cells indicate that FVIII-sensitized Treg cells are 100% suppressive at a ratio of 1:2 responder T cells, compared to 65% suppression with naive Treg cells. *In vivo* adoptive transfer results show expanded Treg cells from both FVIII sensitized and naive mice are able to regulate anti-FVIII immune responses. Encouragingly, Treg cells expanded FVIII-specifically are 80% CD25 + Foxp3 + and have 100% FVIII-specific suppressive activity at a ratio of 1:16 responder T cells, compared to < 20% activity in non-specifically expanded FVIII-sensitized Treg cells.

Conclusion: Our data demonstrate that Treg cells can be successfully expanded *in vitro* and effectively suppress anti-FVIII inhibitor response in HemA mice after FVIII challenge. The FVIII-specific expansion protocol yields even more potent Treg cells to induce long-term tolerance to FVIII, thus significantly increasing the translational potential of adoptive Treg cell therapy.

Disclosure of Interest: None declared.

OR110

Hepatocellular distribution of FVIII in *ex vivo* perfused rat liver: liver sinusoidal endothelial cells are the cells primarily responsible for uptake of FVIII

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Background: It is well established that FVIII is primarily cleared by the liver.

Aims: To determine which liver cells are responsible for uptake and degradation of FVIII, the hepatocellular distribution of FVIII was investigated by immunohistochemistry (IHC) of rat livers perfused *ex vivo* with recombinant FVIII (turoctocog alfa) with or without co-administration of a molar excess of a fragment of von Willebrand Factor (VWF) containing the FVIII binding site (D'D3A1).

Methods: Rat livers were isolated and cannulated via the portal vein and vena cava to generate an *ex vivo* recirculating model. FVIII (2 nM) was added to a Krebs Henseleit/BSA perfusate buffer with or

without VWF-D'D3A1 (35 nM). Thereafter, histological liver sections were stained for FVIII and either CD31 (staining liver sinusoidal endothelial cells (LSECs)) or CD68 (staining Kupffer cells).

Results: Brightfield microscopy of FVIII stained rat livers showed that FVIII was taken up by both hepatocytes and by non-parenchymal cells, consisting of LSECs and Kupffer cells. Non-parenchymal cells stained significantly stronger than hepatocytes. Since hepatocytes are the most abundant cells in the liver, FVIII clearance via these cells may still be significant. The FVIII staining pattern was identical in livers perfused for 20 min and 80 min, demonstrating that FVIII is steadily degraded by all FVIII-uptaking cell types within this time span. Co-formulation of FVIII with VWF-D'D3A1 reduced the liver uptake of FVIII, but the relative distribution of FVIII between cell types was not altered. Double staining for FVIII and non-parenchymal cells revealed that the majority of FVIII was found in CD31-positive LSECs, with weaker staining of Kupffer cells.

Conclusion: FVIII is internalized by all major cell types in the liver, with the strongest FVIII staining detected in LSECs as compared to Kupffer cells and hepatocytes. This suggests that LSECs, in addition to hepatocytes, have a primary role in FVIII clearance.

Disclosure of Interest: K. Roepstorff Shareholder of: Novo Nordisk, Employee of: Novo Nordisk, M. Loftager Shareholder of: Novo Nordisk, Employee of: Novo Nordisk, J. Clausen Shareholder of: Novo Nordisk, Employee of: Novo Nordisk, M. Kjalke Shareholder of: Novo Nordisk, Employee of: Novo Nordisk, F. Rode Employee of: Novo Nordisk, J. Haaning Employee of: Novo Nordisk, R. Appa Shareholder of: Novo Nordisk, Employee of: Novo Nordisk.

Platelets – kinases-I

OR111

Distinct pathways regulate SYK activation downstream of itam and HEMI-ITAM receptors in platelets

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Background: Tyrosine kinase pathways are known to play an important role in the activation of platelets. In particular, GPVI and CLEC-2 receptors are known to activate Syk upon tyrosine phosphorylation of an Immune Tyrosine Activation Motif (ITAM) and hemi-ITAM, respectively. However, unlike GPVI, the CLEC-2 receptor contains only one tyrosine motif in the intracellular domain. The mechanisms by which this receptor activates Syk are not completely understood.

Aims: In this study, we identified a novel signaling mechanism of Syk activation downstream of CLEC-2 hemi-ITAM receptor

Methods: We used pharmacological and genetical approach to evaluate the role of PI3-Kinase and Tec-family kinases downstream of CLEC-2 in platelets. We used pharmacological inhibitors such as LY294003, pan PI3-Kinase inhibitor and Ibrutinib, a Tec-family kinase inhibitor to evaluate the role of these molecules in Syk activation. We also used Btk^{-/-}, Tec^{-/-}, Btk^{-/-} and Tec^{-/-} double knockouts to evaluate the role of Tec kinases in CLEC-2 signaling and Syk activation.

Results: CLEC-2-mediated, but not GPVI-mediated, platelet activation and Syk phosphorylation were abolished by inhibition of PI3-Kinase and Tec-family, which demonstrates that PI3-Kinase and Tec-family kinases regulate Syk downstream of CLEC-2. To address the role of Tec family kinases downstream of CLEC-2 *in vivo* we analyzed Btk^{-/-}, Tec^{-/-} and Btk^{-/-};Tec^{-/-} double-deficient mice embryos to the vascular phenotype that can be usually seen in embryos with

CLEC-2^{-/-} or Syk^{-/-}. Animals lacking Btk or Tec alone exhibited no vascular phenotype, but Btk^{-/-};Tec^{-/-} double-deficient embryos exhibited cutaneous edema associated with blood-filled vessels in a typical lymphatic pattern similar to CLEC2^{-/-} deficient embryos.

Conclusion: Thus our data show, for the first time, that a PI3-Kinase and Tec-family kinases play a crucial role in regulation of platelet activation and Syk phosphorylation downstream of the CLEC-2 receptor.

Disclosure of Interest: None declared.

OR112

Glycogen synthase kinase-3 phosphorylation prevents pathological GPVI-mediated platelet activation and thrombosis

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Background: Glycogen synthase kinase-3 (GSK3) is a constitutively active kinase. Its activity is negatively regulated by phosphorylation of a serine residue near its NH₂-terminus (GSK3 α/β : Ser21/9). We previously demonstrated that PAR-mediated GSK3 phosphorylation supports platelet function. However, the role of GSK3 phosphorylation in GPVI-mediated platelet activation and thrombosis is presently unknown.

Aims: The aim of our study is to identify the role of GSK3 phosphorylation in platelet activation and thrombus formation by the collagen receptor GPVI.

Methods: GPVI-mediated platelet function (aggregation, ATP-secretion, integrin activation (JON/A binding) and P-selectin exposure) and thrombosis (*in vitro* flow studies) were examined using platelets from wild-type (WT) and GSK3 α/β knock-in (KI) mice. GSK3 KI mice express a non-phosphorylatable form (GSK3 $\alpha^{Ser21Ala}$ /GSK3 $\beta^{Ser9Ala}$) of GSK3.

Results: Stimulation of platelets with a range of agonists resulted in phosphorylation of GSK3 α/β at Ser21/9, which was absent in platelets from GSK3 KIs. Expression of total GSK3 α/β was unchanged. Thrombin-mediated platelet activation was reduced in GSK3 KI platelets, confirming our previous findings. In contrast collagen-related peptide (CRP-XL)-mediated platelet activation was dramatically enhanced in GSK3 KI platelets. This occurred with a parallel increase in GPVI signalling, but without significant alterations in platelet GPVI surface expression. Furthermore thrombus formation on a collagen-coated surface was increased when using blood from GSK3 KI mice. Intriguingly inhibition of GSK3 activity with potent and selective ATP-competitive inhibitors (CHIR-99021 and LY2090314) did not significantly alter CRP-XL-mediated platelet activation in both WT and KI.

Conclusion: Collagen-mediated GSK3 α/β phosphorylation prevents pathological platelet activation and thrombus formation in a manner that is independent of GSK3 α/β kinase activity.

Disclosure of Interest: None declared.

OR113

MEKK3 controls the ERK1/2- and JNK2-mediated platelet activation and arterial thrombosis

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Background: Mitogen-activated protein kinases (MAPKs) play important roles in platelet activation and function. It is well known that

MAPKs are activated through three-tiered kinase cascades classically including a MAP3K, a MAP2K and a MAPK. Although MEKK3, a member of the MAP3K family, is an important activator of MAPK pathways, its functions in platelet activation and thrombosis remain unknown.

Aims: To reveal the roles of MEKK3 in platelet activation and thrombus formation.

Methods: Platelet specific MEKK3 deletion mice were developed to study the functions of MEKK3 in platelet activation and thrombosis. FeCl₃-induced carotid artery thrombosis model was used to evaluate the role of MEKK3 in arterial thrombosis *in vivo*. The functions of MEKK3 in platelet activation were assessed by flow-cytometry and aggregometry *in vitro*.

Results: We found that ablation of MEKK3 in platelets resulted in delayed the FeCl₃-induced carotid artery occlusion *in vivo*. Aggregation and degranulation of MEKK3-deficient platelets in response to low levels of thrombin, ADP and Thromboxane A₂ receptor agonist U46619 were markedly reduced. At the molecular levels, MEKK3-deficiency led to severely impaired activation of extracellular signal-regulated kinases 1/2 (ERK1/2) and c-Jun NH₂-terminal kinase 2 (JNK2), but not the MAPKs p38 or ERK5. Using chemical inhibitors for JNKs, ERK1/2, p38 and ERK5, we found that the MEKK3 dependent platelet aggregation in response to ADP, U46619 and proteinase-activated-receptor 4 agonist peptide differentially require ERK1/2 and JNK2, but not ERK5 or p38. However, ERK5 and p38 synergize with the MEKK3 mediated platelet activation, and full activation of platelets requires both the MEKK3-mediated signals and the alternative activation of ERK5 or p38.

Conclusion: Our results unravel a previously undocumented critical role of MEKK3 in platelet activation and thrombus formation. Our study also provides mechanistic support for MEKK3 and ERK5 as new promising antiplatelet targets.

Disclosure of Interest: None declared.

OR114

Casein kinase 2B is critical player in platelet activation, arterial thrombosis and ischemic stroke

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Background: Intracellular signaling upon platelet activation is crucial to development of acute arterial thrombotic occlusions. Casein kinase 2 (CK2) is an ubiquitous expressed tetramer composed of two α and two regulatory β subunits. CK2 is expressed in platelets and becomes activated upon platelet stimulation, but the impact of CK2-dependent signaling on platelet activation is unclear.

Aims: The present study aimed to elucidate the impact of CK2 regulatory β subunit on platelet activation and arterial thrombosis.

Methods: CK2 β -floxed mice were crossed with PF4-Cre mice to generate platelet-specific CK2 β deficient mice (ck2 $\beta^{cre/+}$) and wildtype littermates (ck2 $\beta^{+/+}$); FACS analysis; luminescence measurements; aggregometry; spectrofluorometry; flow chamber; FeCl₃-induced injury of mesenteric arterioles; occlusion of the middle cerebral artery (MCAO); phospho-proteomics.

Results: Activation-dependent platelet secretion as well as integrin $\alpha_{IIb}\beta_3$ activation and platelet aggregation were severely impaired in ck2 $\beta^{cre/+}$ platelets in response to activation with CRP or thrombin. Ca²⁺ measurements pointed to a significant reduction in activation-dependent cytosolic Ca²⁺ increase due to defective Ca²⁺ influx in ck2 $\beta^{cre/+}$ platelets upon stimulation with CRP or thrombin. *In vitro* thrombus formation on a collagen-coated surface under high arterial

shear rates (1700 s⁻¹) and thrombotic vascular occlusion *in vivo* following were significantly diminished in ck2 $\beta^{cre/+}$ mice. Furthermore ck2 $\beta^{cre/+}$ mice are protected against cerebral ischemia when subjected to MCAO. Ck2 $\beta^{cre/+}$ mice displayed drastically reduced cerebral infarct volumes and developed significantly fewer neurological deficits (grip test, Bederson score) following MCAO while tail bleeding time was only mild prolonged in ck2 $\beta^{cre/+}$ mice. Finally, we identified potential downstream targets of CK2 in platelet activation by phospho-proteomic approaches.

Conclusion: The present observations unravel CK2 β as a novel powerful regulator of platelet activation and arterial thrombosis.

Disclosure of Interest: None declared.

OR115

The focal adhesion kinase PYK2 mediates CA2+-dependent activation of the SRC family kinases FYN and LYN in thrombin-stimulated platelets

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Background: In blood platelets, thrombin stimulates the Ca²⁺-dependent activation of Src family kinases (SFKs), resulting in the tyrosine-phosphorylation of multiple substrates, but the mechanism underlying this process is still poorly understood.

Aims: We investigated the involvement of the Ca²⁺-dependent focal adhesion kinase Pyk2 in SFKs activation by thrombin.

Methods: Thrombin- and Ca²⁺-ionophore-induced protein tyrosine phosphorylation was evaluated in platelets from selective knock out (KO) mice.

Results: We found that the time-dependent protein-tyrosine phosphorylation triggered by thrombin in human or murine platelets was totally suppressed only upon concomitant chelation of intracellular Ca²⁺ and inhibition of SFKs. Thrombin-induced activation of SFKs was regulated by intracellular Ca²⁺, and accordingly, the Ca²⁺ ionophore A23187 was sufficient to stimulate SFKs. A23187 also triggered the phosphorylation and activation of Pyk2, and Pyk2 activation by thrombin was Ca²⁺-dependent. Interestingly, stimulation of SFKs by thrombin or A23187 was strongly reduced in platelets from Pyk2 KO, as it was the overall pattern of protein-tyrosine phosphorylation. By immunoprecipitation experiments, we demonstrate that Lyn and Fyn, but not Src, were activated by Pyk2. Inhibition of SFKs by PP2 also reduced the phosphorylation of Pyk2 in thrombin- or A23187-stimulated platelets. Analysis of KO mice demonstrated that Fyn, but not Lyn, was required for maximal Pyk2 phosphorylation by thrombin. Finally, PP2 reduced aggregation of murine platelets to a level comparable to that of Pyk2-deficient platelets, but did not have further effects in the absence of Pyk2.

Conclusion: These results indicate that in thrombin-stimulated platelets, stimulation of Pyk2 by intracellular Ca²⁺ initiates SFK activation, establishing a positive loop that reinforces the Pyk2/SFK axis and allows the subsequent massive tyrosine phosphorylation of multiple substrates required for platelet aggregation.

Disclosure of Interest: None declared.

Stroke

OR116

CM352, a new antifibrinolytic, reduces hematoma growth and improves functional recovery in a collagenase-induced rat model of intracerebral hemorrhage

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Background: Intracerebral hemorrhage (ICH) is an acute neurological disorder with high mortality and no effective treatment. In addition to the initial bleeding event, rebleeding and hematoma expansion are associated with poor outcome in these patients.

Aims: We have studied the effectiveness of the novel antifibrinolytic agent CM352, a short half-life (1.4 h) matrix metalloproteinase (MMP) inhibitor, to achieve early hemostasis and improve functional outcome in a rat model of collagenase-induced ICH.

Methods: ICH was induced by striatal injection of collagenase and, at 1 h later, rats received an intravenous injection of saline ($n = 6$) or CM-352 (1 mg kg⁻¹, $n = 6$). Hematoma (basal, 3 h and 24 h) and lesion (14 days) volumes were quantified on T2-weighted magnetic resonance images (MRI). In order to estimate functional recovery, neurological deficit was evaluated using Bederson score and motor impairment was assessed using the cylinder test, quantified by laterality index (basal, 24 h and 14 days).

Results: CM352 was efficient reducing hematoma expansion early at 3 h (310 ± 62 vs $200 \pm 86\%$; $P < 0.01$) and, more markedly, at 24 h (305 ± 51 vs $133 \pm 63\%$; $P < 0.001$). Less bleeding after antifibrinolytic treatment was associated to smaller lesion volume at 14 days (27.8 ± 4.1 vs 14.4 ± 2.4 mm³; $P < 0.001$). CM352 drastically reduced sensorimotor impairment after ICH in rats from 73% to 16% ($P < 0.01$) at 24 h, and from 49% to 6% ($P < 0.01$) at 14 days. Interestingly, it also attenuated neurological deficit at 24 h (71 vs 14%; $P < 0.01$) and 14 days (43 vs 0%; $P < 0.01$).

Conclusion: CM-352, a new antifibrinolytic agent and MMP inhibitor, effectively prevents hematoma growth and reduces lesion size, associated to better functional and neurological recovery, in a rat model of collagenase-induced ICH.

Disclosure of Interest: None declared.

OR117

Assessment of the diagnostic value of plasma levels, activities and their ratios of von Willebrand factor and ADAMTS13 in patients with cerebral infarction

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Background: Cerebrovascular disease (CVD) is a high morbidity and mortality disease worldwide and cerebral infarction is the most common CVD manifestation. Raised levels of VWF and reduced levels of ADAMTS13 activity are associated with thrombosis.

Aims: Our aim is to investigate the relationships between plasma levels of VWF and ADAMTS13, their ratios, and the occurrence of cerebral infarction.

Methods: Ninety-four cerebral infarction patients and 103 controls were analyzed. Plasma levels of VWF antigen (VWF:Ag), VWF ristocetin cofactor activity (VWF:Rcof), and VWF collagen binding activity (VWF:CB) were measured by ELISA. ADAMTS13 activity (ADAMTS13) was measured with FRET-S-VWF73.

Results: The levels of VWF:Ag and VWF:Rcof in cerebral infarction patients (median [IQR], 176% [114% > 293%] and 197% [105% > 381%]) were significantly higher compared with controls (105% [61% > 145%] and 100% [60% > 175%], $P < 0.001$). Levels of ADAMTS13 and ratios of VWF:CB/VWF:Ag, ADAMTS13/VWF:Ag and ADAMTS13/VWF:Rcof in patients (109% [80% > 132%], 0.43 [0.28–0.90], 0.54 [0.32–0.99] and 0.57 [0.29–0.91], respectively) were significantly lower compared with controls (117% [103% > 146%], 1.13 [0.72–1.81], 1.23 [0.79–2.16] and 1.18 [0.71–1.79], $P < 0.01$, respectively). However, there were no statistical differences between cerebral infarction and controls in VWF:CB, VWF:Rcof/VWF:Ag or ADAMTS13/VWF:CB. The highest quartile of VWF:Ag (OR = 5.11, 95% CI, 1.49–17.50), VWF:Rcof (OR = 5.04, 95% CI, 1.62–15.66), and the lowest quartile of VWF:CB/VWF:Ag (OR = 5.91, 95% CI, 1.95–17.93), ADAMTS13/VWF:Ag (OR = 9.11, 95% CI, 2.49–33.33), ADAMTS13/VWF:Rcof (OR = 3.73, 95% CI, 1.39–10.03) have been found to have an association with cerebral infarction.

Conclusion: Our data suggest that the joint analysis of VWF:Ag, VWF:Rcof, VWF:CB/VWF:Ag, ADAMTS13/VWF:Ag, ADAMTS13/VWF:Rcof are useful to predict the risk of cerebral infarction.

Disclosure of Interest: None declared.

OR118

Is anemia a risk factor for cerebral venous thrombosis?

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Background: Anemia is sometimes considered a risk factor for cerebral venous thrombosis (CVT), but evidence for this assumption is mainly based on small and uncontrolled studies.

Aims: To determine the association between anemia and CVT.

Methods: Case-control study. Cases were adult patients with CVT included a prospective database of consecutive patients admitted to the Academic Medical Center from July 2006 to December 2014. Controls were healthy subjects included in the Dutch MEGA study (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis). Anemia was defined according to WHO criteria: non-pregnant women Hemoglobin (Hb) < 7.5 mmol L⁻¹; pregnant women Hb < 6.9 mmol L⁻¹; men Hb < 8.1 mmol L⁻¹. We used logistic regression analysis to adjust for: age, sex, malignancy, oral contraceptive use, and pregnancy/puerperium.

Results: We included 152 cases and 2916 controls. Four patients with CVT were excluded because of missing baseline Hb. Patients with CVT were younger (mean age 40 vs. 48 years), more often female (74% vs. 53%) and more often had been diagnosed with cancer (9% vs. 4%) compared to controls. Oral contraceptive use was higher among cases (70% vs. 21%). Mean Hb concentration was lower in cases than in controls (8.06 vs. 8.68 mmol L⁻¹, $P < 0.001$). Anemia was more frequent in cases (41/152, 27.0%) than in controls (189/2916, 6.5%, $P < 0.001$). Anemia was associated with CVT, both in univariate analysis (OR 5.33, 95% CI 3.62–7.85) and after adjustment for potential confounders (adjusted OR 4.40, 95% CI 2.82–6.85). Hb (as a continuous variable) was inversely associated with CVT (adjusted OR per 1 mmol L⁻¹ change in Hb 0.52, 95% CI 0.41–0.65). Stratification by sex showed a stronger association between anemia and CVT in

men (adjusted OR 9.92, 95% CI 4.13–23.82) than in women (3.52, 95% CI 2.11–5.86).

Conclusion: Cerebral venous thrombosis is associated with anemia. Whether this is a causal relation remains to be investigated.

Disclosure of Interest: None declared.

OR119

Risk profiles and 1-year outcomes of patients with newly diagnosed atrial fibrillation: results from GARFIELD-AF

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Background: Atrial fibrillation (AF), the most common cardiac arrhythmia, is associated with a high risk of ischemic stroke, systemic thromboembolism, heart failure, and mortality.

Aims: To study risk profiles, anticoagulation (AC) control, and 1-year outcomes in patients with newly diagnosed non-valvular AF.

Methods: Of 17,168 prospective patients were enrolled in 2010–2013 in the GARFIELD-AF global registry. All patients provided written informed consent. Independent ethics committee and hospital-based institutional review board approvals were obtained, as necessary, for the registry protocol. We analyzed 1-year data for baseline characteristics, antithrombotic therapy, and incidence of stroke/systemic embolization (SE), death, and major bleeding.

Results: At diagnosis, 60.8% of patients received AC therapy, 27.4% antiplatelet therapy alone, and 11.8% no antithrombotic therapy. Rates of death, stroke/SE, and major bleeding were 4.1, 1.4, and 0.8 per 100 person-years, respectively. The baseline characteristics associated with increased mortality and stroke/SE, respectively, were age (≥ 75 vs. < 65 year hazard ratio [HR] 4.3, 2.3), prior stroke/transient ischemic attack (HR 1.6, 1.8), presence of diabetes (HR 1.3, 1.3), CHA₂DS₂-VASc score (6–9 vs. 0–1 HR 7.4, 4.5), and HAS-BLED score (3–9 vs. 0–1 HR 3.9, 3.1). In patients with CHA₂DS₂-VASc ≥ 2 , poor vitamin K antagonist (VKA) control (time in therapeutic range $< 60\%$; HR 2.2, 1.9), missing international normalized ratio (HR 3.4, 1.6), and no AC therapy (HR 3.2, 2.4) were associated with a higher risk of mortality and stroke/SE than patients with adequate VKA control or on a non-VKA AC.

Conclusion: One-year mortality and stroke/SE in newly diagnosed AF are associated with high CHA₂DS₂-VASc score, high HAS-BLED

score, advanced age, no AC use, poor AC control, prior stroke/transient ischemic attack, and diabetes.

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OR120

Red cell distribution width is associated with future risk of incident stroke: the Tromsø Study

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Background: Red cell distribution width (RDW), an easy measure of variability in size of circulating erythrocytes, is associated with cardiovascular morbidity and mortality. However, the association between RDW and stroke has never been explored in a general population.

Aims: To investigate whether RDW was associated with incident stroke and case fatality in a large prospective cohort with subjects recruited from the general population.

Methods: Baseline characteristics, including RDW, were obtained from 25,992 subjects participating in the Tromsø Study. Incident stroke was registered from inclusion (1994/95) until December 31st 2010. Cox regression models were used to calculate hazard ratios (HR) with 95% Confidence Intervals (95% CI) for stroke, adjusted for age, sex, body mass index, smoking, hemoglobin level, white blood cell

count, thrombocyte count, hypertension, total cholesterol, triglycerides, self-reported diabetes, and red blood cell count. The study was approved by the regional ethical committee, and informed written consent was obtained from all participants.

Results: There was 1152 participants that experienced a first-ever stroke during a median follow-up of 15.8 years. A 1% increment in RDW yielded a 13% higher risk of stroke (multivariable HR: 1.13, 95% CI: 1.07–1.20). Subjects with RDW in the highest quintile (RDW > 14.3%) compared to the lowest (RDW 10.7–12.3%) had a 37% higher risk of stroke in multivariable analysis (HR: 1.37, 95% CI: 1.11–1.69). Subjects with RDW above the 95-percentile had 55% higher risk of stroke compared to those in the lowest quintile (HR: 1.55, 95% CI: 1.16–2.06). All risk estimates remained unchanged after exclusion of subjects with anemia ($n = 1102$). RDW was not associated with increased risk of death within one year or during the entire follow-up after an incident stroke.

Conclusion: RDW is associated with incident stroke in a general population, independent of anemia and traditional atherosclerotic risk factors.

Disclosure of Interest: None declared.

Innate and Acquired Immunity

OR121

Neutrophil extracellular trap (NET) formation in patients with post-burn sepsis

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Background: Major thermal injury results in immune dysfunction increasing the risk of infection and sepsis. Central to host defence and innate immunity are neutrophils. An emerging concept in neutrophil biology is neutrophil extracellular traps (NETs). NETosis is the extrusion of nuclear material to the exterior of the cell resulting in a network of fibres that ensnare bacteria. NETosis results in post translational modifications of multiple proteins which are potential novel biomarkers of the onset of infection e.g. citrullinated histone H3 (Cit H3).

Aims: As NETosis is important both in infection and sepsis we hypothesized that NETs are involved in either the host immune response to burn injury and/or during the pathogenesis of sepsis.

Methods: Blood samples were routinely collected from 39 patients with burn injury (day 1–12 months following injury). Sepsis was defined as an American Burns Association Sepsis Score ≥ 3 with evidence of microbiological infection. Plasma cell-free DNA (cfDNA) levels were analysed using a Sytox[®] Green fluorometric assay. Cit H3 was analysed by SDS-PAGE and Western blotting in plasma samples ($n = 15$).

Results: Septic patients ($n = 19$) have elevated cfDNA levels ($P < 0.0001$) during septic episodes compared to healthy controls ($n = 10$). Maximal change in cfDNA levels from baseline were significantly elevated in septic patients ($P < 0.0001$, $n = 19$, median change in DNA $809.28 \text{ ng mL}^{-1}$) compared to non-septic patients ($n = 20$, median change 45.94 ng mL^{-1}). Cit H3 was detectable in septic patient's plasma corresponding with elevated cfDNA levels thus confirming NETosis. Cit H3 was absent from non-septic patients and healthy controls. Preliminary data suggests that isolated neutrophils from septic patients ($n = 5$) have reduced *ex vivo* NET generation compared to neutrophils from healthy controls.

Conclusion: NETosis occurs following human thermal injury and elevated levels of cfDNA are associated with septic episodes. Addition-

ally, sepsis results in neutrophil dysfunction resulting in reduced *ex vivo* NET generation.

Disclosure of Interest: None declared.

OR122

Compared to B220/CD45R+ B lymphocytes, splenic dendritic cells and macrophages have limited propensity to bind factor VIII and demonstrate down-regulation of co-stimulatory molecules

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Background: Hemophilia A treatment is severely complicated in 25–30% of patients by the formation of neutralizing antibodies against infused factor VIII (FVIII). The fate and cellular interactions of FVIII remain unclear. A previous study identified that FVIII accumulates in the marginal zone of the spleen, a region abundant in professional antigen-presenting cells (APCs).

Aims: To assess the binding and maturation of splenic antigen presenting cells in response to FVIII.

Methods: Hemophilia A C57Bl/6 mice were intravenously infused with 30 IU recombinant human FVIII (FVIII). Splenic co-stimulatory molecule expression was assessed by flow cytometry. To assess binding, naïve splenocytes were cultured in the presence of 1 IU mL^{-1} FVIII for 1 h and labeled for flow cytometry.

Results: In a mouse with a near 100% inhibitor risk, the percentage of CD11c+ myeloid dendritic cells (DCs) expressing co-stimulatory molecules CD40, CD80, and CD86 were shown to decrease by 2.6%, 3.3%, and 3.6% respectively ($P = 0.01$, 0.06 , 0.02 , $n = 3$) 48 h following FVIII infusion. Similarly, we observed a 6.4%, 7.2% and 7.3% decrease in CD40, CD80, and CD86 respectively ($P = 0.01$, 0.01 , 0.02 , $n = 3$) in CD11b+ macrophages and monocytes. Cell association assays indicated that approximately 5% of splenic lymphocytes bind FVIII *in vitro* ($n = 3$). DCs and macrophages showed limited binding capacity for FVIII, with only 2.1% and 2.8% co-localization with FVIII respectively. More intriguingly, we detected substantial co-localization of FVIII with B220+ cells (4.5%).

Conclusion: Decreases in maturation and co-stimulatory molecules, as well as minimal FVIII binding suggest that DCs and macrophages do not contribute to the initiation of the anti-FVIII immune response. In contrast, significant levels of FVIII co-localization with B220+ cells suggests that this population, predominantly splenic B cells, may play a significant role in FVIII processing and immunity.

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OR123

Mitochondrial DNA and toll-like receptor 9 are associated with mortality in critically ill patients

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Background: Despite underlying pathologies leading to ICU admittance are heterogeneous, many patients develop a systemic inflammatory response syndrome (SIRS) often in the absence of microbial

pathogens. Mitochondrial DNA (mtDNA) showing similarities to bacterial DNA, may be released after tissue damage activating the innate immune system by binding to toll-like receptor- (TLR)-9 on immune cells.

Aims: The aim of this study was to analyze whether levels of mtDNA are associated with 30-day survival and whether this predictive value is modified by TLR-9 expression.

Methods: In this single-center, prospective, observational trial, 228 consecutive patients admitted to a medical ICU at a tertiary care center were enrolled. Blood was taken within 24 h after ICU admission and levels of circulating mtDNA were quantified by rtPCR and TLR-9 expression on monocytes was measured by FACS.

Results: Median APACHE II was 20 (13–25) and 30-day mortality was 25%. Median mtDNA levels at admission were significantly higher in non-survivors as compared to survivors (26.9 IQR 11.2–60.6 ng mL⁻¹ vs. 19.7 IQR 9.5–34.8 ng mL⁻¹, $P < 0.05$). Patients with plasma levels of mtDNA in the highest quartile (mtDNA > 38.2 ng mL⁻¹) had a 2.6-fold higher risk ($P < 0.001$) of dying, independently of age, gender, diagnosis and APACHE II score. mtDNA improved the c-statistic of APACHE II ($P < 0.05$) and showed enhancement in individual risk prediction indicated by a net reclassification improvement of 32.3% ($P < 0.05$). Stratification of patients according to TLR-9 expression above/below median demonstrated that only patients with high expression of TLR-9 showed an increased risk associated with increased mtDNA levels (OR 2.7; $P < 0.01$) whereas circulating mtDNA was not associated with mortality in patients with low TLR-9 expression (OR 1.1; $P = 0.98$).

Conclusion: Circulating levels of mtDNA at ICU admission predict mortality in critically ill patients. This association was in particular present in patients with elevated TLR-9 expression.

Disclosure of Interest: None declared.

OR124

AKI development upon SIRS is associated with platelet activation, possibly triggered by mitochondrial DNA derived from damaged cells

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Background: Multiple inflammatory pathways are activated by non-infectious agents during systemic inflammatory response syndrome (SIRS) leading to collateral damage, including the development of acute kidney injury (AKI). Pattern recognition receptors (PRRs) of the innate immune system sense Damage-Associated Molecular Patterns (DAMPs) leading to an immune response. Components of mitochondria e.g. unmethylated CpG-enriched mitochondrial DNA (mtDNA) that leak upon cell injury, are a source of DAMP. Platelets express several PRRs and play an important role in innate immunity. To date, it is unclear which intrinsic factors trigger the onset of SIRS and associated AKI.

Aims: We investigated the role of mtDNA during systemic inflammation and AKI and the role of platelets herein.

Methods: Human platelets were stimulated with mitochondrial DNA, genomic DNA or unmethylated CpG-enriched oligonucleotides (CpG ODN). Platelet activation was measured with FACS. In a single centre prospective observational study we included 37 patients diagnosed and classified for the onset of SIRS with or without the development of AKI that were admitted to the ICU. As a control we included ($n = 25$) patients after elective major surgery without a diagnosis of either SIRS or AKI. Blood and urine samples were isolated and processed. Free circulating plasma and urinary mtDNA was determined by RT-PCR. Inflammatory mediators and PF4 in urine were measured using specific ELISA.

Results: Mitochondrial DNA, but not genomic DNA triggered platelet activation. Unmethylated CpG ODN, dose dependently stimulated platelet activation. Compared to ICU-controls, critically ill patients with SIRS demonstrated elevated levels of plasma and urinary mtDNA and proinflammatory cytokines. Compared to SIRS patients without AKI, the patients with AKI displayed elevated levels of urinary PF4 –indicative of platelet activation.

Conclusion: The development of AKI in critically ill patients is associated with platelet activation, possibly triggered by mitochondrial DNA.

Disclosure of Interest: None declared.

OR125

Diphosphorylated lipid A triggers tissue factor mRNA splicing in human platelets

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Background: Lipopolysaccharide (LPS) is a known agonist for platelet messenger ribonucleic acid (mRNA) splicing such as processing of tissue factor (TF) and interleukin-1 β (IL-1 β) pre-mRNA. However, it is uncertain which molecular component of LPS might be important in inducing splicing processes.

Aims: Investigating which structural LPS component is critical for platelet mRNA splicing.

Methods: Isolated and CD45-depleted platelets were treated with LPS, its derivatives lipid A di- and monophosphate or 3-deoxy-D-manno-2-octulosonic acid (KDO). As a control, platelets were incubated with PBS or dimethylsulfoxide (DMSO) as vehicle. In addition, platelet mRNA processing was analyzed at baseline in quiescent platelets. Platelet RNA was isolated, converted into cDNA, and mRNA splicing of TF and IL-1 β was assessed using PCR and subsequent separation by agarose gel electrophoresis.

Results: LPS and lipid A diphosphate but not lipid A monophosphate or KDO triggered efficient mRNA splicing. Whereas TF mRNA was not processed at baseline and was not or only slightly spliced after incubation with PBS or DMSO, expression of spliced IL-1 β mRNA was already detectable without any further treatment.

Conclusion: Platelets seem to be more susceptible in processing IL-1 β mRNA as compared to TF mRNA splicing. Analyzing TF mRNA splicing provides an appropriate tool to investigate which LPS component mediates platelet mRNA splicing. The diphosphorylated lipid A moiety of LPS is sufficient to trigger splicing processes. In summary, this study supports the role of platelets in bridging innate immunity and thrombosis.

Disclosure of Interest: None declared.

Cancer and thrombosis – clinical – I

OR126

Risk of cardiovascular events associated with BCR-ABL TKIs: a systematic review and meta-analysis

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Background: Clinical trials have demonstrated a high incidence of vascular occlusive events associated with the use of ponatinib. Peripheral arterial occlusive events were also reported with nilotinib. To date, no

vascular safety signals were found with imatinib, dasatinib and bosutinib.

Aims: To assess the link between tyrosine kinase inhibitors (TKIs) and vascular occlusive events to recommend, if needed, risk minimization measures concerning their use.

Methods: Studies were selected by two independent reviewers from the PubMed, Scopus and Cochrane Library databases. Selected studies include all randomized controlled trials (RCTs), whatever the disease, comparing a BCR-ABL TKI vs. the reference therapy. Two investigators independently extracted data on study design, patient characteristics, primary efficacy endpoints, overall survival and vascular occlusive events. The meta-analysis was performed using both the random-effect and the fixed-effect model. Odd ratios with 95% confidence intervals were computed.

Results: Seventeen RCTs were selected for the meta-analysis. Risk of vascular occlusive events was increased with nilotinib (ORPETO: 3.51; 95% CI 2.09–5.89) and ponatinib (ORPETO: 3.47; 95% CI: 1.23–9.78) compared to imatinib in patients with Ph+ chronic myeloid leukemia. No significant difference was found with bosutinib (ORPETO: 7.48; 95% CI: 0.15–376.94) and dasatinib (ORPETO: 2.65; 95% CI 0.88–8.03) compared to imatinib. Novel generation TKIs increased the rate of major molecular response at 1 year compared to imatinib in the treatment of Ph+ chronic myeloid leukemia. However, there was no statistical difference in term of overall survival at 1 year.

Conclusion: Nilotinib and ponatinib increase the risk of vascular occlusive events. Risk minimizations should be proposed for these two compounds. Novel generation TKIs improve major molecular response at one year but not the overall survival.

Protocol registration number: PROSPERO 2014:CRD42014014147

Disclosure of Interest: None declared.

OR127

Differences in venous thromboembolic events in subjects enrolled in standard phase I clinical trials and those on phase I hepatic dysfunction clinical trials

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Background: Venous thromboembolic events (VTEs) are a significant cause of death in patients with cancer and they significantly reduce short-term prognosis. The incidence of VTEs is not well characterized in patients in early phase clinical trials of novel antineoplastic agents or in hepatic dysfunction studies test escalating doses of antineoplastic agents in patients with varying degrees of hepatic abnormalities. Patients with hepatic dysfunction may have limited ability to synthesize coagulation factors and accordingly may experience fewer VTEs.

Aims: We sought to evaluate the incidence of VTEs in phase I clinical trials and hepatic dysfunction trials sponsored by the National Cancer Institute of the United States.

Methods: We reviewed the records of 1896 subjects: 1359 subjects enrolled on 42 separate phase I clinical trials, and 537 subjects enrolled on 9 separate hepatic dysfunction clinical trials. Symptomatic VTEs that were diagnosed while on study were abstracted and compared between the groups using a two-sided Fisher's exact test. The Common Terminology Criteria for Adverse Events was used to report severity of VTEs.

Results: There were 43 VTEs identified amongst the 1896 subjects (2.3%). There were significantly more VTEs in the subjects on phase I clinical trials ($n = 38$, 2.8%) than in the subjects on hepatic dysfunction studies ($n = 5$, 0.9%; $P = 0.015$). For subjects on phase I clinical

trials, VTEs usually occurred on the second cycle (median) of treatment [interquartile range (IQR) 1–4], were grade 3 (median; IQR 3–4) in severity, and were associated with the final cycle 42% of the time. For subjects on hepatic dysfunction studies, VTEs usually occurred on the second cycle of treatment (median; IQR 1–5), were grade 3 (median, IQR 2–3) in severity, and were associated with the final cycle 60% of the time.

Conclusion: VTEs are less common in patients on hepatic dysfunction studies than those on standard phase I clinical trials.

Disclosure of Interest: None declared.

OR128

Risk factors for incident venous thromboembolism in active cancer patients: a population based case-control study

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Background: Independent risk factors for cancer-associated incident venous thromboembolism (VTE) are uncertain.

Aims: To identify non-cancer and cancer-specific risk factors for cancer-associated incident VTE.

Methods: Using Rochester Epidemiology Project resources, we identified all Olmsted County, MN residents with active cancer identified between 1973 and 2000 who had objectively diagnosed incident VTE, excluding arm DVT (cases; $n = 570$), and matched controls on age, sex, duration of active cancer, date of and duration cancer ($n = 604$). We reviewed their complete medical records for non-cancer baseline characteristics previously identified as independent VTE risk factors and cancer-specific characteristics. Using conditional logistic regression, we tested non-cancer and cancer-specific variables, including the effect of cancer site on VTE using a previously-published score, for association with VTE.

Results: Compared to controls, VTE cases had a higher cancer site score, cancer grade, stage and liver metastasis prevalence, more often received chemotherapy, radiation therapy and progesterone, were hospitalized, underwent surgery or received anesthesia more frequently, and had a higher prevalence of neurological disease, renal disease, autoimmune connective tissue disease, immobilization, trauma/fracture, pacemaker/central venous catheter (CVC), prior superficial vein thrombosis, and infection. On multivariate analysis, higher cancer site score (OR = 1.4 per 2-fold increase), higher cancer stage (OR = 2.1), liver metastasis (OR = 2.6), stage progression (OR = 1.6) and chemotherapy (OR = 1.7) were independently associated with VTE, as were hospitalization (OR = 7.2), nursing home confinement (OR = 6.7), CVC (OR = 6.3) and any recent infection (OR = 1.6).

Conclusion: Cancer site, advanced cancer stage, liver metastasis, stage progression, chemotherapy, hospitalization/nursing home confinement, CVC, and infection are independent risk factors for incident VTE in cancer patients.

Disclosure of Interest: None declared.

OR129

Cohort profile: the Scandinavian Thrombosis and Cancer (STAC) Study

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Background: Venous thromboembolism (VTE) is a common complication in cancer patients, and further knowledge about high-risk patients who may benefit from thromboprophylaxis is needed. We have established the prospective Scandinavian Thrombosis and Cancer (STAC) Study by merging individual data from three large Scandinavian cohorts (The Tromsø Study, the HUNT Study and the Danish Diet, Cancer and Health Study).

Aims: To explore the cohort profile of the STAC Study, provide age-specific incidence rates of cancer and VTE and compare the cancer incidence rates with national figures.

Methods: The STAC Study includes 14,4952 subjects aged 19–101 years without previous cancer or VTE. Baseline information including physical examination, self-administered questionnaires and blood samples was collected in 1993–1997, and cancer and VTE were registered up to 2007–2012. Cancer information was obtained from complete national cancer registries. Only objectively confirmed symptomatic VTEs were included. Regional committees of research ethics approved the study, and participants gave their informed written consent.

Results: In total, 19,757 subjects developed cancer during follow-up (9.76 per 1000 person-years), and the 5-year age-specific incidence rates of cancer were comparable with corresponding rates from the Norwegian Cancer Registry. There were 2444 VTE events (1.45 per 1000 person-years) during follow-up, and the incidence rate increased from 0.28 per 1000 person-years in subjects aged 20–29 years to 6.44 per 1000 person-years in subjects aged 80+. Overall, 51% of the VTE events were provoked, and cancer was the most common provoking factor (19%), followed by immobilization and surgery (both 15%).

Conclusion: The STAC Study is a large cohort with validated events of cancer and VTE and with age-specific incidence rates similar to national figures. Future studies will provide a unique opportunity to explore genetic and environmental risk factors for cancer-related VTE in the general population.

Disclosure of Interest: None declared.

OR130

Prediction of venous thromboembolism in lung cancer patients receiving chemotherapy

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Background: There is a clear association between cancer and venous thromboembolism (VTE). However, primary thromboprophylaxis is challenging as VTE risk varies with type of cancer, patient co-morbidity, and chemotherapy; and in some patients may do more harm than good. The Khorana VTE risk score has been validated for use in a heterogeneous cancer population but has not been evaluated in a single cancer primary.

Aims: To evaluate the utility of the Khorana score in predicting the risk of developing VTE in lung cancer patients receiving chemotherapy.

Methods: Patients with primary non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) within 6 weeks of diagnosis and prior to commencing anticancer treatment were enrolled in the FRAGMATIC study. Patients were randomised in a 1:1 fashion to active thromboprophylaxis (dalteparin 5000 IU daily, 24 weeks) or no thromboprophylaxis (control group). All patients were risk assessed using the Khorana score and evaluated against VTE outcome data.

Results: The control group was comprised of 910 lung cancer patients that received chemotherapy. Mean age 65% and 60% were male. Eighty-five percent of patients had an ECOG performance status of 0–1. Of the NSCLC patients 94% were stage III and IV and 64% of SCLC had limited disease. A total of 108/910 (11.9%) lung cancer patients receiving chemotherapy developed VTE during the 52 week study period. The rate of VTE for Khorana risk score 1, 2, 3 and 4 were 44/342 (12.9%), 35/322 (10.9%), 26/225 (11.6%) and 3/21 (14.3%) respectively.

Conclusion: In a large population of lung cancer patients receiving chemotherapy, the current components of the Khorana score are unable to discriminate between patients at high and low risk of VTE. There is a need to further develop cancer specific thrombosis risk assessment tools to help guide targeted thromboprophylaxis.

Disclosure of Interest: S. Noble Grant/Research Support from: Pfizer, Leo Pharma, Consultant for: Pfizer, BMS, Leo Pharma, Boehringer Ingelheim, A. Robbins: None declared, R. Alikhan Consultant for: Pfizer, BMS, Leo Pharma, Boehringer Ingelheim, K. Hood: None declared, F. Macbeth: None declared.

Venous thromboembolism – epidemiology – II

OR131

Risk of venous thrombosis in individuals using vitamin supplements: a case-control study

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Background: Whether vitamin supplements decrease venous thrombosis risk is controversial. Previous reports did not all take confounding fully into account, either by randomization or extensive adjustment.

Aims: The aim of our study was to determine whether vitamin supplementation decreases the risk of venous thrombosis.

Methods: A large case-control study included 2506 patients with venous thrombosis, 2506 partner controls and 2684 random digit dialing controls (RDD). When patients were compared with RDD controls, unconditional logistic regression was used to calculate odds ratios (ORs) with 95% confidence intervals (CIs). When patients were compared with partner controls, conditional logistic regression was used, providing further adjustment for unmeasured confounding.

Results: Vitamin use yielded a 37% lower risk of venous thrombosis than no vitamin use (OR 0.63; 95% CI 0.57,0.70), when comparing patients with RDD controls. Adjustment for several putative confounders did not change the estimate (OR 0.68; 95% CI 0.61, 0.77). The fully adjusted ORs for vitamin A, vitamin B-6, vitamin B-12, folic acid, vitamin C, vitamin D, vitamin E, and multivitamin use were in the same range. However, when patients were compared with partner controls, ORs attenuated to unity. Results were similar for provoked and unprovoked events, and for deep vein thrombosis and pulmonary embolism.

Conclusion: After extensive adjustments, vitamin supplementation was no longer associated with a decreased risk of venous thrombosis in this study. Previous positive results may have been spurious due to uncontrolled confounding.

Disclosure of Interest: B. Vučković Grant/Research Support from: Dr Biljana Vuckovic was supported by Isth Reach the World Fellowship for year 2013., N. van Rein: None declared, S. Cannegieter: None declared, F. Rosendaal: None declared, W. Lijfering: None declared.

OR132

The incidence of chronic thromboembolic pulmonary hypertension and the predictive value of residual arterial pulmonary obstruction: results from a large, multicentre, prospective cohort study

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Background: The true incidence of chronic thromboembolic pulmonary hypertension (CTEPH) after an acute episode of pulmonary embolism (PE) is still not well known. A high 3.8% incidence was observed in a previous cohort study. Whether residual arterial pulmonary obstruction (RPO) increases the risk of recurrent venous thromboembolism (VTE) and CTEPH is unknown.

Aims: To determine the risk of recurrent VTE and CTEPH over a 3-year follow-up in a wide cohort of PE patients.

Methods: In a nationwide, multicentre, prospective cohort study, consecutive patients with first, objectively confirmed acute PE were prospectively followed for up to 3 years. Patients with severe cardiopulmonary diseases, low expected survival or refusal to provide informed consent were excluded. A perfusional lung scan was performed after 6 months and an echocardiogram was performed after 6 week and 6 months. RPO was assessed using a residual perfusion

defects index. Recurrent VTE and CTEPH were assessed locally and an independent central adjudication will follow.

Results: Six hundred and fifty-two out of 706 pts enrolled in the study were followed for 3 years and received a lung scan at 6 months. The duration of anticoagulant therapy was 20.8 ± 12.8 months. Pulmonary perfusion defects were found in 326 patients (50%). Recurrent VTE developed in 49 patients (7.5%), 32 (9.8%) in the group with RPO and 17 (5.2%) in that without RPO ($P = 0.0259$). The value for CTEPH was 10 (1.5%), all belonging to the group with RPO (3.1%) ($P = 0.0014$).

Conclusion: The incidence of CTEPH after first acute PE is lower than that observed in previous studies. RPO, as assessed by lung scan 6 months after an episode of PE, is a predictor of CTEPH and recurrent VTE.

Disclosure of Interest: None declared.

OR133

Cross-sectional associations of 25-hydroxyvitamin D with hemostatic and inflammatory biomarkers in the Multi-Ethnic Study of atherosclerosis

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Background: Vitamin D deficiency has been associated with the risk of arterial cardiovascular disease (CVD) and venous thrombosis (VT).

Aims: As mechanisms may include an influence of vitamin D on hemostasis and inflammation, we hypothesized that serum concentrations of 25-hydroxyvitamin D (25-OHD) would be associated with biomarkers of both.

Methods: In the Multi-Ethnic Study of Atherosclerosis, 9 hemostatic biomarkers (D-dimer, fibrinogen, factor VIII, Plasmin-antiplasmin [$n = 6443$]; von Willebrand factor, soluble tissue factor, PAI-1, total TFPI and soluble thrombomodulin [$n = 814$]) and 3 inflammatory biomarkers (IL-6, CRP [$n = 6443$]; TNF-alpha soluble receptor [$n = 3802$]) were measured among U.S. adults free of CVD. We estimated cross-sectional associations of 25-OHD with log-transformed biomarker concentrations using linear regression, adjusting for age, race/ethnicity, site, education, income, physical activity, BMI, waist circumference, diabetes, renal function, use of statin, hormone therapy and aspirin.

Results: Our sample comprised 6443 subjects (46.6% men, mean age and BMI of 62.1 years and 28.3 kg m^{-2}), of White (37.8%), Black (27.2%), Asian (12.2%) or Hispanic (21.8%) race/ethnicity. Mean 25-OHD levels were 25.3 ng mL^{-1} (SD 10.9). After adjustment, 25-OHD was strongly associated with IL-6 and marginally with PAI-1 and TFPI [per 10 ng mL^{-1} decrement in 25-OHD: increment of 5.1% of IL-6 (95% CI 3.4–6.9, $P < 0.001$); increment of 7.0% of PAI-1 (95% CI 0.9–13.6, $P = 0.025$); increment of 2.1% of TFPI (95% CI 0.0–4.2, $P = 0.047$), without racial/ethnic effect modification. No associations were observed for other hemostatic and inflammatory biomarkers.

Conclusion: Our findings do not suggest a meaningful influence of 25-OHD deficiency on hemostasis, apart from a possible inhibition of fibrinolysis as reflected by increased PAI-1 levels. Increased inflammation through IL-6 levels may represent a mechanism linking vitamin D deficiency to greater risks of arterial CVD and possibly VT.

Disclosure of Interest: None declared.

OR134

Unprovoked deep vein thrombosis is associated with future risk of disability pensionBraekkan S^{1,2}, Grosse S³, Okoroh EM⁴, Tsai J⁴, Cannegieter SC⁵, Naess IA⁶, Krokstad S⁷, Hansen J-B^{1,2} and Skjeldestad F-E⁸¹Thrombosis Research and Expertise Center (TREC), Department of Clinical Medicine, UiT – The Arctic University of Norway;²Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway; ³National Center on Birth Defects and Developmental Disabilities; ⁴National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention, Atlanta, USA; ⁵Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands; ⁶Department of Hematology, Trondheim University Hospital, Trondheim; ⁷HUNT Research Centre, Department of Public Health and General Practice, Norwegian University of Science and Technology, Levanger; ⁸Research Group Epidemiology of Chronic Diseases, Department of Community Medicine, UiT – The Arctic University of Norway, Tromsø, Norway**Background:** The burden of venous thromboembolism (VTE) related to permanent work-related disability has never been assessed among a general population.**Aims:** To estimate the risk of work-related disability in subjects with incident VTE compared with those without VTE in a cohort recruited from the general population.**Methods:** From the Tromsø Study and the Nord-Trøndelag Health Study (HUNT), Norway, 66 005 individuals aged 20–65 years were enrolled in 1994–1997 and followed to December 31, 2008. Incident VTE events among the study participants were identified and validated, and information on work-related disability was obtained from the Norwegian National Insurance Administration database. Cox-regression models using age as time-scale and VTE as a time-varying variable were used to estimate hazard ratios (HR) with 95% confidence intervals (CI) adjusted for age, sex, BMI, smoking, education level, marital status, history of cancer, diabetes, cardiovascular disease and self-rated general health.**Results:** During follow-up, 384 subjects had a first VTE and 9862 participants were granted disability pension. The crude incidence rate of work-related disability after VTE was 37.5 (95% CI: 29.7–47.3) per 1000 person-years, vs. 13.5 (13.2–13.7) per 1000 person-years among those without VTE. Subjects with unprovoked VTE had a 52% higher risk of work-related disability than those without VTE (HR 1.52, 95% CI 1.09–2.14), after multivariable adjustment; the association appeared to be driven by deep vein thrombosis.**Conclusion:** VTE was associated with subsequent work-related disability in a cohort recruited from the general working-age population. Our findings suggest that indirect costs due to loss of work time may add to the economic burden of VTE.**Disclosure of Interest:** None declared.

OR135

Sex difference in the risk of venous thrombosis: a detailed analysis in four European cohortsRoach R¹, Lijfering W¹, Tait RC², Baglin T³, Kyrle P⁴, Cannegieter S¹ and Rosendaal F¹¹Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands; ²Department of Haematology, Glasgow Royal Infirmary, Glasgow; ³Department of Haematology, Addenbrooke's Hospital, Cambridge, UK; ⁴Department of Medicine I, Medical University of Vienna, Vienna, Austria**Background:** Previous analyses reported a higher risk of recurrent venous thrombosis in men than in women.**Aims:** We aimed to assess the recurrence risk in men compared with women whilst taking female reproductive risk factors (i.e. oral contraception, postmenopausal hormone therapy and pregnancy) into account. In addition, we hypothesized that the sex-difference in venous thrombosis risk was related to *F9* Malmö: an X-linked prothrombotic factor.**Methods:** In four pooled European cohort studies (the AUREC study, Vienna, Austria; CVTE study, Cambridge, United Kingdom; CAR-ROT study, Glasgow, United Kingdom; and LETS follow-up study, Leiden, the Netherlands), the risk of recurrent venous thrombosis was calculated in men, women with reproductive risk factors and women without reproductive risk factors at the time of their first venous thrombosis. *F9* Malmö was genotyped, and carriers and non-carriers contrasted.**Results:** A total of 2185 patients with a first venous thrombosis, 1043 men and 1142 women, were included in this study. Overall, men had a 2.8-fold (95% CI, 2.2–3.4) higher risk of recurrent venous thrombosis than women. This risk was 5.2-fold (95% CI, 3.5–7.7) higher in men than in women with reproductive risk factors, and 2.3-fold (95% CI, 1.7–3.2) higher in men than in women without reproductive risk factors. No difference in recurrence risk was found for carriers vs. non-carriers of *F9* Malmö.**Conclusion:** In this study of four European cohorts, men experienced a recurrent venous thrombosis twice as often as women without reproductive risk factors. These findings indicate that men have a higher intrinsic risk of venous thrombosis than women which is partly masked by female reproductive risk factors. The sex difference cannot be explained by the X-linked prothrombotic factor, *F9* Malmö.**Disclosure of Interest:** None declared.**Immune thrombocytopenia – basic**

OR136

The defective IL-21/IL-21R pathway in the B lymphocytes of patients with immune thrombocytopeniaZhang D, Wenjie L, Huiyuan L, Xian Z, Feng X and Renchi Y
State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China**Background:** Primary immune thrombocytopenia (ITP) is an autoimmune disease accompanied by skewed T1 polarization and auto-reactive B lymphocytes. IL-21 can regulate the activation, proliferation and differentiation of human B cell and immunoglobulin production as well as isotype switching of plasma cell.**Aims:** This study aimed to investigate the role of IL-21/IL-21R pathway played in ITP.

Methods: Serum IL-21 concentrations were measured by ELISA. The percentages of IL-21R and activation-associated surface markers (CD25 and CD69) expression on B cells, and the frequency of circulating IL-21 producing T-cells in peripheral blood (PB) were analyzed by flow cytometry. Peripheral blood mononuclear cells (PBMC) were stimulated by rhIL-21, and changes of IL-21R, activation-associated surface markers, the proliferation and apoptosis of B cells were analyzed by flow cytometry. Production of IgG in the culture supernatants was determined by ELISA.

Results: The results showed that the serum IL-21 levels in ITP patients were significantly higher than that of healthy controls (HC), and it had correlation with disease status. IL-21R, CD25 and CD69 expression on B cells and the frequency of circulating CD4⁺ IL-21⁺ and CD8⁺ IL-21⁺ T-cells were significantly higher in ITP patients. *In vitro* cultures, exogenous IL-21 downregulated IL-21R expression, up-regulated activation-associated surface markers on B cells, promoted more B cell proliferation and apoptosis in ITP patients than healthy controls. This IL-21-mediated effect could be reversed by IL-21R-specific neutralizing antibody. Meanwhile, IL-21 promoted higher levels of IgG production in ITP patients than healthy controls.

Conclusion: The defective IL-21/IL-21R pathway in the B lymphocytes may be involved in the pathogenesis of ITP.

Disclosure of Interest: None declared.

OR137

Soluble costimulatory factors SB7-H2, SB7-H3 in relation to platelet autoantibodies in patients with immune thrombocytopenic purpura

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Background: Immune thrombocytopenia (ITP) is a common bleeding disorder, which is mainly attributed to immunopathogenic processes including platelet auto-antibodies mediated platelet destruction and impaired platelet production. The B7 family molecules expressed mainly on B cell are critical for T cell regulation and antibody production.

Aims: This paper aims to investigate whether the plasma levels of platelet auto-antibodies in ITP patients are related to that of co-stimulatory molecules sB7-H2 and sB7-H3.

Methods: A total of 61 ITP patients and 25 healthy controls from the First Affiliated Hospital of Soochow University during June 2012 and August 2013 were enrolled. The expression levels of platelet auto-antibodies against five glycoproteins (GPIX, GPIb, GPIIIa, GPIIb and P-selectin) in plasma were detected by flow cytometric immuno-beads array, and the expression of soluble co-stimulatory molecules sB7-H2 and sB7-H3 was measured by ELISA.

Results: Plasma levels of five auto-antibodies against platelet membrane glycoproteins were higher in ITP patients ($P < 0.01$). Compared with healthy controls, sB7-H2 levels increased ($P < 0.05$), while there was no significant difference with sB7-H3. In ITP patients, platelet counts were negatively correlated to sB7-H2 ($r = -0.3907$, $P < 0.01$), but had no correlation with sB7-H3. However, The level of sB7-H3 was significantly negatively correlated to that of platelet P-selectin auto-antibody ($r = -0.46$, $P < 0.05$).

Conclusion: Soluble costimulatory molecules sB7-H2 was elevated in ITP patients, and the level of sB7-H3 was associated with auto-antibodies against P-selectin, which suggests that costimulatory molecules B7-H2 and B7-H3 may be involved in the pathogenesis of immune regulation of ITP.

Disclosure of Interest: None declared.

OR138

High-dose dexamethasone corrects impaired myeloid-derived suppressor cell functions via positive crosstalk between glucocorticoid receptor and Ets1 in immune thrombocytopenia

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Background: Myeloid-derived suppressor cells (MDSCs) are heterogeneous immature cells and natural inhibitors of adaptive immunity.

Aims: To define MDSC's role in immune thrombocytopenia (ITP).

Methods: In this study, the MDSCs population was determined in patients with primary ITP before and after high-dose dexamethasone (HD-DXM) treatment.

Results: Our data demonstrated that both the numbers and suppressive functions of CD33⁺CD11b⁺HLA-DR⁻ MDSCs were impaired in peripheral blood and the spleen of ITP patients compared with healthy controls. HD-DXM promoted suppressive functions of MDSCs induced from human PBMCs and splenocytes, exerting optimal inhibition of CD4⁺ T cell proliferation at a concentration of 20 nM. Moreover, the expression of IL-10 and TGF- β was up-regulated significantly in DXM-modulated MDSCs compared with the unmodulated systems. After HD-DXM treatment in ITP patients, MDSC-induced autologous T regulatory cells Tr1 increased significantly. When co-cultured with MDSC-primed CD8⁺ T cells, the platelet apoptosis level became lower, suggesting the enhanced control over CTL-mediated platelet lysis. Elevated expression of glucocorticoid receptor (GR) and transcription factor Ets1 was demonstrated in DXM-modulated MDSCs. Transfection of Ets-1 siRNA efficiently suppressed MDSC functions, which almost offset the effect of HD-DXM. Splenocytes from CD61 knockout mice immunized against CD61(+) platelets were transferred into severe combined immunodeficient (SCID) (C57/B6 background) mouse recipients to induce a murine model of severe ITP within 3 weeks. At the same time we passively transferred the DXM-modulated MDSCs induced from bone marrow of WT C57/B6 mice into the SCID mouse recipients, which showed significantly raised platelet counts compared with mice receiving non-modulated MDSCs or the blank ITP model.

Conclusion: These findings suggested that impaired MDSCs may be involved in the pathogenesis of ITP, and that HD-DXM could correct their functions via positive crosstalk between GR and Ets1.

Disclosure of Interest: None declared.

OR139

Suppression of cell-mediated immune thrombocytopenia (ITP) by B cell depletion therapy in a murine model

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Background: Immune thrombocytopenia (ITP) is a bleeding disorder that is mediated by both anti-platelet antibodies (Ab⁺) and CD8⁺ T cells (Ab⁻). One therapy for ITP is B cell depletion (Bdep) by Rituximab, an anti-CD20 antibody which appears to prevent Ab⁺ platelet destruction. Recently, however, studies have shown that Ab⁻ ITP patients also respond to Bdep, indicating an Ab-independent mechanisms may exist.

Aims: To investigate the effects of anti-CD20 Bdep therapy in murine ITP.

Methods: To establish murine Ab⁻ ITP, splenocytes from CD61 knockout mice (β 3⁻) immunized against β 3⁺ platelets were collected,

depleted of CD19 + B cells *in vitro*, and 3×10^4 cells were transferred into $\beta 3^-$ severe combined immunodeficient (SCID) mice and weekly platelet counts monitored. For Bdep *in vivo*, anti-mouse CD20 was administered to $\beta 3^-$ mice before immunization (250 μ g per mouse, iv). *In vitro* proliferation of purified CD8 + T cells was assessed after 72 h anti-CD3/anti-CD28 stimulation. *In vivo* proliferation was assessed by CD8 + T percentages in SCID mouse spleens three weeks after T cell injections into SCID mice.

Results: Anti-CD20 induced significant Bdep in the blood (> 98%), spleen (> 90%) and lymph nodes (> 80%) of $\beta 3^-$ mice. Compared with immunized non-depleted mice, splenocytes from Bdep mice could not induce ITP in SCID mice. Similarly, purified CD8 + T cells from the Bdep $\beta 3^-$ mice showed decreased proliferation *in vitro* and could not induce ITP *in vivo* as compared with T cells from the non-depleted mice.

Conclusion: Bdep by α CD20 showed a therapeutic effect in Ab- ITP that correlated with suppressed proliferation of CD8 + T cells suggesting that B cell depletion therapy may significantly affect CD8 + T cell responses.

Disclosure of Interest: None declared.

OR140

STAT1 single nucleotide polymorphisms and susceptibility to immune thrombocytopenia

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Background: Primary immune thrombocytopenia (ITP) is an acquired autoimmune bleeding disorder. In ITP patients, IFN- γ is significantly up-regulated as a result of Th1 polarization. One of the key mediators of IFN- γ signaling is the Signal Transducer and Activator of Transcription 1 protein (STAT1).

Aims: To evaluate the relationship between *STAT1* gene single nucleotide polymorphisms (SNPs) and the associated risk of ITP in a prospective case-control study.

Methods: A total of 548 children were recruited: 328 children with ITP and 220 healthy children as sex- and age-matched normal controls. Sequenom MassArray system was used to detect three SNPs genotypes in the *STAT1* gene: rs10208033, rs12693591 and rs1467199.

Results: There is a statistically significant difference in *STAT1* rs1467199 allele frequencies with comparison of each of the four clinical subgroups of ITP patients to the normal controls ($P = 0.0432$). Also, newly diagnosed ITP patients and chronic ITP patients demonstrate significant different genotypes ($\chi^2 = 8.511$, $P = 0.0142$) and allelic frequency ($P = 0.0055$). Although a positive *STAT1* rs1467199 genotype subgroups to *STAT1* mRNA expression level cannot be established, there is a weak correlation between *STAT1* mRNA level and the activity ratio of Type 1 T helper lymphocyte and Type 2 T helper lymphocyte (Th1/Th2 ratio) ($P = 0.0544$); Correlation with IFN- γ alone did not reach statistical significance ($P = 0.1715$). Si Also, there is no significant difference of platelet count among the 3 subgroups: rs10208033, rs12693591 and rs1467199 ($P = 0.1646$, $P = 0.7524$, $P = 0.0731$, respectively).

Conclusion: The findings in our study suggest *STAT1* rs1467199 SNP plays a potential role in the IFN- γ dependent development of autoimmunity in children with ITP. The important clinical implication of *STAT1* SNPs testing as a predictor of pediatric chronic ITP will be validated in future molecular and protein functional analysis.

Disclosure of Interest: None declared.

Hemophilia Inhibitors

OR141

Do vaccinations influence the risk of inhibitor development in patients with severe hemophilia A?

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Background: Inhibitor development in previously untreated patients (PUPs) with severe hemophilia A is a multifactorial event. However, it is unknown whether pediatric vaccinations given in combination with FVIII can increase the risk for inhibitors.

Aims: To investigate whether vaccinations given around the time of FVIII exposure increases inhibitor development in PUPs with severe hemophilia A.

Methods: Out of 604 PUPs with severe hemophilia A (FVIII < 1%), we included the 375 patients who had data on any vaccinations between the first and 75th exposure day (ED) to FVIII and before inhibitor development. 229 PUPs were excluded because no data regarding vaccinations between the 1st and 75th ED was available. Inhibitor risk was compared between patients who did and did not receive vaccinations in close proximity to FVIII (48 h before to 24 h after). Both the associations between inhibitor development and any vaccinations and the number of vaccinations were assessed. Unadjusted and adjusted hazard ratios (HR) were calculated using Cox regression.

Results: Inhibitors developed in 79 out of 375 patients (21.1%), high titer inhibitors in 46 (12.3%), after a median of 7.4 months (interquartile range 4.3–11.6). This was different from the entire cohort, where inhibitors developed in 30.6% (20.4% high titer) after 3.6 months (IQR 1.2–7.6). There were 1662 vaccinations given and 232 PUPs (61.9%) received at least one vaccination concomitant with FVIII. No association between vaccinations in combination with FVIII exposure and inhibitor development was found: adjusted HR 0.74 (95% CI 0.58–1.16) for all inhibitors, 0.88 (0.48–1.59) for high titer inhibitors. This association was independent of the number of vaccinations.

Conclusion: We found no significant association between vaccinations given in close proximity to FVIII exposure and inhibitor development. Our study suggests that there is no reason to time vaccinations to be given far apart from FVIII exposure.

Disclosure of Interest: None declared.

OR142

Estimation of APTT improvement due to MC710 administration in hemophilia patients with inhibitorsNakano H¹, Hayashida K², Imamura M², Tomokiyo K¹, Ohashi Y³ and Shirahata A⁴¹Blood Plasma Division, Business Promotion Department;²Clinical Development Department, Kaketsuken, Kumamoto;³Integrated Science and Engineering for Sustainable Society, Chuo University, Tokyo; ⁴Kitakyushu Yahata Higashi Hospital, Kitakyushu, Japan

Background: MC710, a mixture product of human plasma-derived activated factor VII (FVIIa) and factor X (FX) at a protein weight ratio of 1:10, is a novel bypassing agent for hemostasis in hemophilia patients with inhibitors. MC710 contains both FVIIa and its substrate FX for greater potency than FVIIa alone, and is long acting due to the long half-life of FX. We had completed 3 clinical trials (Phase I-III) targeting hemophilia patients with inhibitors and MC710 was approved in Japan in July, 2014 (brand named *Byclot*®).

Aims: There is no standardized objective index for hemostasis monitoring in bypassing agent treatment at present. It is important to find an index related to MC710 administration.

Methods: We re-analysed the pharmacokinetics (PK) and pharmacodynamics (PD) parameters of MC710, obtained in the Phase I clinical trial, and estimated the dose-response of MC710 and the correlation of the PK/PD parameters.

Results: Both the AUC and C_{max} of FVIIa and FX increased in a dose-dependent manner after administration of MC710, and indicated linearity in the range of 20–120 µg kg⁻¹. Under the PD parameters, APTT showed a dose-response from 10 min to 24 h after administration. However, PT and each parameter of clot waveform analysis and the thrombin generation test did not indicate a dose-response to MC710. Therefore, we performed linear regression analysis with a curve fitting on three parameters (APTT, administration dosage and elapsed time from administration) and found an approximate equation. The estimated APTT value calculated from this approximate equation was compared with the actual measured APTT value from the Phase II clinical trial, and showed little difference.

Conclusion: These results suggest that approximate APTT can be predicted from the administration dosage of MC710 and elapsed time from administration. As further treatment evidence by MC710 is accumulated, it will become possible to predict treatment with MC710 using APTT as an objective index.

Disclosure of Interest: H. Nakano Employee of: Kaketsuken, K. Hayashida Employee of: Kaketsuken, M. Imamura Employee of: Kaketsuken, K. Tomokiyo Employee of: Kaketsuken, Y. Ohashi Consultant for: Kaketsuken, A. Shirahata Consultant for: Kaketsuken.

OR143

Plasma-derived factor VIII products and inhibitor development in previously untreated boys with severe hemophilia A: report of the FranceCoag NetworkGoudemand J^{1,2}, Rothschild C³, d'Oiron R⁴, Demiguel V⁵, Dalibard V^{1,5}, Micheau M⁶, Lutz P⁷, Gruel Y^{8,9}, Vinciguerra C^{10,11}, Chambost H^{12,13}, Calvez T^{14,15} and on behalf of FranceCoag Network¹Haematology Unit, University Hospital; ²Université Lille2, Lille;³Hemophilia Center, AP-HP, Necker, Paris; ⁴Hemophilia Center,AP-HP, Bicêtre, Le Kremlin Bicêtre; ⁵French Institute for PublicHealth Surveillance, Saint Maurice; ⁶Hemophilia Center,University Hospital, Bordeaux; ⁷Pediatric Hematology OncologyUnit, Hautepierre University Hospital, Strasbourg; ⁸HaematologyUnit, University Hospital; ⁹Université François Rabelais, Tours;¹⁰Haematology Unit, Hospices Civils; ¹¹EA 4174, UniversitéLyon1, Lyon; ¹²Pediatric Hematology Oncology Unit, la TimoneUniversity Hospital; ¹³Aix-Marseille University, Marseille;¹⁴Sorbonne Universités, Université Pierre et Marie Curie 06,UMR 1136; ¹⁵INSERM, UMR 1136, Institut Pierre Louis

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Background: It has been suggested that inhibitor risk is greater in severe hemophilia A (HA) patients first treated with recombinant (r) products than in those treated with plasma derived (pd) concentrates. In most studies it is postulated that rFVIII constitutes an homogeneous group. Recently 3 independent studies reported an increased inhibitor risk in previously untreated patients (Pups) treated with second (2nd)-generation vs. third (3rd) generation full-length rFVIII products. On the other hand, pdFVIII concentrates have also distinct features precluding global analysis.

Aims: To compare the inhibitor incidence rates in Pups treated with a pdFVIII concentrate and those treated with 2nd or 3rd generation FVIII concentrates in the FranceCoag (FC) Pups cohort.

Methods: The FC network has been funded by the public health authorities and comprises a prospective national multicenter Pups cohort (34 centers). Parents approve their child's enrollment in accordance with the Declaration of Helsinki. Baseline data comprised demographic characteristics, HA-related medical history from birth, F8 gene defect, family history of H and inhibitor, ethnic origin. Detailed H treatments and major events were collected at each follow-up visits such as the results of FVIII inhibitor assays. Only the first 75 exposure days were considered for this analysis. Three outcomes were considered: inhibitor development (> 0.6 Bethesda Unit), high titer inhibitor (≥ 5 BU) and the use of by passing agents or immune tolerance induction.

Results: Among the 508 eligible Pups with severe HA (FVIII < 1%) enrolled on January 26th 2015, 376 were first treated with rFVIII (1st generation: 72, 2nd generation: 170, 3rd generation: 134) and 116 were first treated with pdFVIII (including 106 treated with the same pdFVIII concentrate). The inhibitor incidence in Pups treated with pdFVIII compared to the different generation of rFVIII will be presented.

Conclusion: Will be presented later.

Disclosure of Interest: None declared.

OR144

Determinants of inhibitor development in previously treated hemophilia A patients

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Background: The development of inhibitors, or neutralizing alloantibodies, is the most serious complication in hemophilia A treatment. The assessment of the risk for inhibitor formation in new or modified factor concentrates is traditionally performed in previously treated patients (PTPs). Inhibitor development is a complex process affected by multiple issues and risk factors, including non-modifiable risks (e.g. genetics) and environmental risks (e.g. treatment and trigger events such as surgery, immunologic challenge, trauma).

Aims: The purpose of this study is to examine cases of de novo inhibitors in PTPs reported in the scientific literature and to the European Haemophilia Safety Surveillance (EUHASS) program and explore determinants of inhibitor development.

Methods: We used a case-series study design. We identified cases via: a) a systematic review of prospective or retrospective cases in the published literature; b) a survey of cases reported to the EUHASS project. We used a case report form (CRFs) to extract the relevant patient level data; including details of inhibitor detection and testing, inhibitor course and treatment, factor VIII products used, and details of events that can trigger inhibitor development (surgery, vaccination, immune disorders, malignancy, product switch).

Results: We identified 19 publications that reported 39 new inhibitors in PTPs with hemophilia A and 26 qualifying inhibitor cases from 19 EUHASS centers. Individual patient data has been collected for 46/65 (70%) inhibitor cases: 13 from CRFs completed by study investigators and 16 extracted from patient-level information available in the published literature and 17 from EUHASS. Data collection will be completed by March and analysis by April 2015. Descriptive analyses of the case series will be presented.

Conclusion: This is the largest cohort of inhibitor development in PTPs assembled to date. Our findings will allow us to explore factors related to inhibitor development in PTPs.

Disclosure of Interest: A. Iorio: None declared, A. Barbara: None declared, G. Castaman: None declared, K. Fischer: None declared, E. Gilman: None declared, J. Goudemand: None declared, K. Kavakli: None declared, C. Kubicek-Hofmann: None declared, T. Lambert: None declared, T. Lissitchkov: None declared, M. Makris: None declared, P. Matthew: Employee of: Bayer, M. E. Mingot: Castelano: None declared, N. Ozdemir: None declared, R. Parra: None declared, K. Peerlinck: None declared, G. Rivolta: None declared, V. Romanov: Employee of: Baxter Healthcare, V. Roussel-Robert: None declared, A. Tagliaferri: None declared, J. Windyga: None declared, E. Zanon: None declared.

OR145

ITI for inhibitory antibodies in boys with severe haemophilia A at eight UK children's centres 2003–2013

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Background: Inhibitory antibodies to FVIII arise in approximately a third of boys with severe haemophilia A and are difficult and costly to manage.

Aims: To document ITI outcome in an unselected consecutive cohort of boys with severe haemophilia A managed at 8 UK centres and to compare these historical data with an on-going audit against recently published UKHCDO guidelines.

Methods: Data were collected by 8 centres from all boys with inhibitors diagnosed between 2003 and 2013.

Results: Sixty-four inhibitor patients with severe haemophilia A received ITI in this period. At detection 44 (68.8%) were < 10BU, 15 (23.4%) were > 10BU and in 5 (7.8%) the inhibitor titre was unknown due to inhibitor evolution occurring outside the UK. At detection median exposure days and inhibitor titre were 15.5 (3–100) and 2.8BU (0.6–119) respectively, historical peak titre 6.5BU (0.6–256) and inhibitor titre at start of first ITI 3.3BU (0.6–95). Ethnicity of the cohort: Caucasian 45 (70.3%), Afro-Caribbean 13 (20.3%), Asian 4 (6.3%) and Other 2 (3.1%). Outcome: tolerance (negative Bethesda, normal recovery and measureable trough FVIII with < 50 IU kg⁻¹ every 48 h) in 31 (48.4%), partial tolerance (negative Bethesda, normal recovery and a measureable trough FVIII with > 50 IU kg⁻¹ every 48 h) in 24 (37.5%) and failure in 9 (14.1%). Tolerance/partial tolerance was achieved with first line ITI in 49 of 64 boys (76.5%), 2nd line in 3 of 14 (21.4%) and 3rd line in 3 of 11 (27.3%). 43/45 (95.6%) of Caucasian boys tolerised compared with 7/13 (53.8%) of Afro-Caribbean boys. 2/44 (4.5%) with < 10BU at diagnosis and 7/15 (46.7%) with > 10BU at presentation failed ITI. This failed group had between 2 and 4 attempts at ITI before switching to prophylaxis with bypassing agents. 42/64 (65.7%) started first line ITI according to the intensity advised by current UKHCDO guidelines.

Conclusion: Tolerance was achieved in 85.9% of this cohort, poor outcome being associated with presenting titre > 10BU and Afro-Caribbean race.

Disclosure of Interest: None declared.

Risk factors for venous thrombosis – I

OR146

Risk factors of residual perfusion defects after a first episode of unprovoked PE: results from the PADIS PE multicenter, double-blind, randomized trial

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Background: After a first episode of unprovoked pulmonary embolism (PE), the presence of residual perfusion lung defects may be associated with a high risk of recurrent venous thromboembolism (VTE) after anticoagulation is discontinued. Risk factors for unresolved PE remain unknown.

Aims: To identify risk factors of residual perfusion defects after a first unprovoked PE.

Methods: In the 'PADIS-PE' multicenter, randomized, double-blind, controlled trial, 371 patients who had completed 6 months of anticoagulation for a first unprovoked PE were allocated to receive an additional 18 months of warfarin or placebo. At the time of inclusion, all patients had a systematic ventilation perfusion (V/Q) lung scan. The pre-determined cut-off for 'residual perfusion defects' was the presence of an altered perfusion in at least 10% of the pulmonary vascular bed. The influence of clinical, biochemical and morphological variables at the diagnosis of PE was determined in univariate and multivariate analyses using logistic regression.

Results: After 6 months of anticoagulation, 124 of 371 patients (33%) had residual perfusion defects. In the multivariate analysis, the Odds Ratio for residual perfusion defects was 3.44 (95% Confidence Interval [CI], 1.97–5.99) for age > 65 years, 2.15 (95% CI, 1.12–4.11) for

patients with a chronic respiratory disease, 1.78 (95% CI, 1.00–3.14) for a systolic pulmonary arterial pressure > 50 mmHg or a right ventricular/left ventricular ratio > 0.9 at the diagnosis of PE and 0.56 (95% CI, 0.32–0.97) for patients who received initial treatment with low molecular weight heparins (LMWH) as compared to unfractionated heparins or pentasaccharide.

Conclusion: Age > 65 years, chronic respiratory disease, and initial right ventricular dysfunction were significantly associated with an increased risk of residual perfusion lung defects, whereas the initial use of LMWH was associated with a decreased risk of residual defects. (ClinicalTrials.gov number NCT00740883).

Disclosure of Interest: None declared.

OR147

Evolutionary conservation of autoinhibitory B-domain regions in blood coagulation factor V of the suborder Serpentes

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Background: The factor V (FV) B-domain negatively regulates FV activity through conserved autoinhibitory regions. The single snake FV sequence identified thus far lacks these inhibitory segments, suggesting a divergent molecular mechanism of FV regulation. Whether this unique feature is limited to the Elapidae or hallmarks the entire Serpentes suborder is unclear.

Aims: We aimed to uncover the identity of the FV B-domain from 18 distinct snake species belonging to 5 snake families and representing different geographical origins and venomous states.

Methods: For 13 species, cDNAs were generated from fresh-frozen liver tissue, and B-domain fragments were amplified employing oligonucleotides complementary to conserved FV-specific sequences. For 5 species, the FV transcripts were compiled from deposited gDNA/cDNA fragments.

Results: Sequence analysis revealed that the regulatory B-domain elements are preserved with high sequence identity in all snake species. All B-domains comprise a proteolytic site homologous to human Arg1018 that likely facilitates removal of the autoinhibitory regions during FV activation. In addition to the ~2 kbp fragment encoding a regular B-domain, the amplification reactions also generated a shorter ~600 bp fragment, corresponding to a truncated FV B-domain that lacks the autoinhibitory regions and is homologous to that found in snake venom FV of several elapids. Alignment with the compiled snake FV transcripts uncovered two intra-exonic splice sites within the B-domain-encoding exon that may be the basis of the truncated B-domain transcript. Preliminary quantitative analysis indicated that while in most snakes the regular B-domain is more abundant (40–1000-fold) than the short version, they are almost equally expressed in snake venom FV-comprising elapids.

Conclusion: Taken together, B-domain-specific structural elements important to FV function occur in all Serpentes families. Alternative splicing events may account for distinct FV B-domain transcripts that vary in composition and abundance.

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OR148

Identification of new genetic risk factors for recurrent venous thrombosisde Haan HG¹, Germain M², Baglin TP³, Deleuze J-F⁴, Trégouët D-A², Rosendaal FR^{1,5,6} and van Hylckama Vlieg A¹

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Background: Previous studies have shown that the established genetic risk factors for a first venous thrombosis (VT) have little effect on the risk of recurrence. So far, genome wide genetic studies on recurrence are lacking.

Aims: Therefore, the aim of this study was to identify genetic determinants of recurrent VT.

Methods: We performed a genome wide association scan in 1279 patients with venous thrombosis from the MEGA follow-up study; 832 patients with a first VT without recurrence, and 447 patients who experienced a recurrent VT during 8 years of follow-up. Genotypes for single nucleotide variants (SNVs) were imputed using the sequencing data of the Genome of the Netherlands (GoNL) project as a reference. We analysed dosages of about 8.6 million SNVs with a minor allele frequency $\geq 1\%$ and an imputation quality $I \geq 0.5$ for an association with recurrent venous thrombosis assuming an additive model of inheritance and adjusted for age and sex.

Results: One region exceeded genome-wide significance ($P \leq 5 \times 10^{-8}$), which mapped to the well-known FV Leiden locus. Conditional association analyses on FV Leiden did not yield secondary association signals at this locus. We also identified 71 highly suggestive association signals ($P < 1 \times 10^{-5}$) at 18 additional loci. None of these loci were previously implicated in VT risk. We are currently genotyping the sentinel SNVs at these loci in 360 recurrent VT patients and 1891 patients with a single VT event from MEGA, THE VTE study, and LETS. Results of the replication efforts will be available at time of the conference.

Conclusion: This is the first large genetic study on recurrent venous thrombosis. Our results reconfirm the association of FV Leiden with recurrent VT. In addition, we identified several new candidate loci that may be associated with the risk of recurrent VT. For these loci replication and further studies are warranted.

Disclosure of Interest: None declared.

OR149

Risk factors of recurrent venous thromboembolism in patients with a first episode of unprovoked pulmonary embolism: results from the PADIS PE multicenter, double-blind, randomized trialCouturaud F¹, Sanchez O², Presles E³, Salaun PY⁴, Pernod G⁵, Mismetti P⁶, Jégou P⁷, Duhamel E⁸, Provost K⁹, Solier CBD¹⁰, Castellant P¹¹, Parent F¹², Bressollette L¹³, Girard P¹⁴, Lacut K¹, Méléac S¹⁵, Guégan M¹⁶, Laporte S³, Leroyer C¹, Décousus H¹⁷, Meyer G² and Mottier D¹

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Background: Patients with a first episode of unprovoked pulmonary embolism (PE) have a high risk of recurrent venous thromboembolism (VTE) after anticoagulation is discontinued.

Aims: To identify risk factors for recurrent VTE after a first episode of unprovoked PE.

Methods: In the 'PADIS-PE' multicenter, randomized, double-blind, controlled trial, 371 patients who had completed 6 months of anticoagulation for a first episode of unprovoked PE were allocated to receive an additional 18 months of warfarin or placebo and were followed during two years after study treatment had been stopped. At the time of inclusion, all patients had biochemical testing, a V/Q lung scan (altered perfusion in $\geq 10\%$ of the pulmonary vascular bed), an echocardiography and a leg ultrasound. The influence of clinical, biochemical and morphological variables was determined using univariate and multivariate analyses adjusted on treatment allocation (Cox model).

Results: During a median study period of 41 months, recurrent VTE occurred in 67 of 371 patients (20.0%; 6.8 events per 100 person-years). In the multivariate analysis, the Hazard Ratio for recurrence was 2.23 (95% Confidence Interval [CI], 1.18–4.21) for age > 65 years, 3.36 (95% CI, 1.16–9.75) for patients who had previous cancer resolved for > 2 years before the diagnosis of PE (none had recurrent cancer at the time of recurrent VTE), and 2.30 (95% CI, 1.22–4.33) for the presence of residual perfusion defects on V/Q scans. Patients with at least one of these factors represented 76% of all recurrences. There was no detectable influence of sex, post-thrombotic syndrome at inclusion, right heart dysfunction at diagnosis of PE or at inclusion, D-dimer level at inclusion and thrombophilia.

Conclusion: In patients with first unprovoked PE, age > 65 years, persistent lung perfusion defects at 6 months of anticoagulation and a previous history of cancer were associated with an increased risk of recurrent VTE. (ClinicalTrials.gov number NCT00740883).

Disclosure of Interest: None declared.

OR150

Lipid levels and risk of venous thrombosis: results from the MEGA study

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Background: Studies on the association between lipids and venous thrombosis (VT) are inconsistent, and the pathophysiology behind this possible link is unclear.

Aims: To assess whether lipids are associated with VT and to explore the mechanism with particular attention to confounding by common risk factors and mediation via hemostatic factors and C-reactive protein (CRP).

Methods: From a case-control study (MEGA study), 2234 patients with a first VT and 2873 controls were included. Total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), non-HDL, triglycerides (TG), and apolipoproteins A1 (apo A1) and B (apo B) were assessed. Consent and ethical approval were obtained for this study.

Results: In age and sex-adjusted models, low levels (< 10th percentile of controls) of apo A1 and HDL increased VT risk as compared with levels > 90th percentile of controls: odds ratios (OR) 2.06 (95% CI 1.58–2.67) and OR 1.64 (95% CI 1.26–2.13), respectively. With further adjustment for body mass index (BMI), the effect of apo A1 on VT risk was attenuated (OR 1.62, 95% CI 1.23–2.12), and the effect of HDL disappeared (OR 1.09, 95% CI 0.83–1.45). Full adjustment (age, sex, BMI, estrogen-, statin- use, and diabetes) did not further change either OR. High levels (> 90th percentile) of apo B decreased VT risk as compared with levels < 10th percentile, even after full adjustment (OR 0.54, 95% CI 0.42–0.71). TC, LDL, non-HDL, and TG were not associated with VT. Although apo A1 and apo B were strongly associated with several hemostatic factors and CRP, their inclusion in the logistic model did not affect the ORs for apo A1 or apo B.

Conclusion: Low apo A1 increased VT risk, and high apo B seemed to protect against VT, which was unexpected as apo B is a risk factor for arterial thrombosis. We were unable to clarify the mechanism by which apo A1 and apo B affect VT risk, but the association of both apolipoproteins with hemostatic factors and CRP may support further studies.

Disclosure of Interest: None declared.

TTP and PNH – from Basic Science to Clinical Practice

OR151

Recombinant ADAMTS13 as an effective therapy for acquired thrombotic thrombocytopenic purpura in rats

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Background: The metalloprotease ADAMTS13 regulates the size of von Willebrand factor (VWF) multimers. A deficiency in ADAMTS13 activity is associated with the life-threatening disorder thrombotic thrombocytopenic purpura (TTP). The vast majority of patients suffer from acquired TTP, where circulating anti-ADAMTS13 autoantibodies are causative for the decreased ADAMTS13 activity. Current treatment consists of plasma exchange, but improved therapies are highly warranted.

Aims: To establish a rat model for acquired TTP and to investigate the therapeutic efficacy of recombinant human (rh)ADAMTS13.

Methods: Rats (Sprague-Dawley) were injected with polyclonal goat anti-ADAMTS13 IgG to inhibit endogenous rat ADAMTS13 activity. Rats were subsequently challenged with recombinant human VWF (rhVWF) to trigger TTP symptoms. The efficacy of rhADAMTS13 to treat acquired TTP in these rats was studied by subsequent injection rhADAMTS13.

Results: Although anti-ADAMTS13 antibodies completely blocked endogenous rat ADAMTS13 activity, the rats did not spontaneously develop TTP. Only when these rats were triggered with rhVWF, the animals developed acquired TTP displaying severe TTP symptoms such as thrombocytopenia, hemolytic anemia and VWF-rich thrombi in kidney and brain. Treatment of acquired TTP was subsequently done by injection of 400, 800 or 1600 U kg⁻¹ rhADAMTS13. Analysis of plasma samples demonstrated the formation of circulating anti-ADAMTS13/rhADAMTS13 immune complexes. However, rhADAMTS13 was able to override circulating anti-ADAMTS13 inhibitory antibodies, resulting in a restoration of ADAMTS13 activity and degradation of ultra-large VWF multimers. As a consequence, rats were rescued from acquired TTP.

Conclusion: We have established a small laboratory animal model for acquired TTP. We demonstrate that rhADAMTS13 is an effective therapy for acquired TTP in rats and holds promising value for future clinical use for the treatment of this severe and life-threatening disease.

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OR152

Hereditary TTP – a young patient population with high prevalence of arterial thromboembolic events: first results from the hereditary TTP registry

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Background: Hereditary TTP or Upshaw-Schulman syndrome is a rare, recessively inherited thrombotic microangiopathy due to congenital ADAMTS13 deficiency caused by *ADAMTS13* mutations.

Aims: To increase the knowledge on hereditary TTP and eventually improve patient care we initiated an international ongoing cohort study for patients diagnosed with hereditary TTP and their family members (ClinicalTrials.gov NCT01257269).

Methods: All patients diagnosed with hereditary TTP are eligible for study. Medical data are recorded retrospectively up to enrollment and then prospectively during follow-up visits and/or at every acute TTP event. Available for study were the validated data sets of 90/106 (85%) patients enrolled until the end of 2014.

Results: As of January 20th 2015, 83/90 (92%) hereditary TTP patients from 82 families were alive. Their median age was 18.7 years (range 0–66.9) and the female to male ratio 1.1. In the 82 families, we identified 79 different *ADAMTS13* mutations [type of mutation: missense 45; nonsense 9; frameshift due to small deletions or insertions 18; large deletion 1; splice site 6] with 4143insA being the most frequent (29 alleles), followed by R193W (10) and R1060W (6). Thirty patients were homozygous and 60 compound heterozygous mutation carriers.

A total of 195 TTP events were recorded in 57 patients and occurred in 61% in the presence of recognized triggers, e.g. infections or pregnancy. Fifty of 195 (26%) events were reported in 15 patients on regular plasma prophylaxis. Despite the young age of the study population, 21/83 (25%) patients had experienced at least one arterial thromboembolic event (TIA, stroke, myocardial infarction, etc.) and one a venous thromboembolic event.

Conclusion: Analysis of the first 90 patients revealed a high prevalence of arterial thromboembolism in hereditary TTP. More than half of TTP events occurred in the context of recognized triggers and the high proportion of events under plasma prophylaxis calls for optimization of current preventive therapy.

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OR153

Endogenous plasmin levels control the development of acute episodes of thrombotic thrombocytopenic purpura in mice

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Background: A deficiency in ADAMTS13 activity is associated with the life-threatening disorder thrombotic thrombocytopenic purpura (TTP), where patients suffer from episodes of thrombotic microangiopathy. We previously demonstrated that exogenous plasmin is able to rescue *Adamts13*^{-/-} mice from TTP implying that plasmin may act as a backup mechanism for ADAMTS13 mediated digestion of VWF.

Aims: Elucidation of the role of endogenous plasmin in the control of TTP episodes in mice.

Methods: ADAMTS13 or PAI-1 activity was blocked using monoclonal antibodies in either *uPAR*^{-/-}, *α2-antiplasmin*^{-/-} or wild type (WT) mice. TTP was triggered using recombinant human (rh)VWF. *In vivo* cleavage of platelet decorated VWF strings was visualized in FeCl₃ injured mesenteric venules, after labeling platelets with Rhodamine 6 G.

Results: WT mice with blocked ADAMTS13 activity developed acquired TTP when triggered with 500 U kg⁻¹ rhVWF, demonstrated by severe thrombocytopenia ($158 \pm 77 \times 10^3$ plt μL^{-1} ; $685 \pm 42 \times 10^3$ plt μL^{-1} at baseline, $n = 15$), while 250 U kg⁻¹ rhVWF did not induce TTP in these animals ($541 \pm 67 \times 10^3$ plt μL^{-1} , $n = 10$). Interestingly however, *uPAR*^{-/-} mice without ADAMTS13 activity did develop TTP when triggered with 250 U kg⁻¹ rhVWF ($209 \pm 44 \times 10^3$ plt μL^{-1} , $n = 10$). Furthermore, VWF string cleavage in these mice was strongly delayed to 18.9 ± 2.7 s compared to 8.8 ± 0.9 s in WT mice. In contrast, VWF string cleavage was accelerated to 5.1 ± 0.2 s in *α2-antiplasmin*^{-/-} mice with inhibited PAI-1 and ADAMTS13 activity. In addition, the latter mice did not develop TTP upon triggering with 500 U kg⁻¹ rhVWF ($569 \pm 267 \times 10^3$ plt μL^{-1} , $n = 16$).

Conclusion: Endogenous plasmin is able to cleave VWF. Blocking plasmin generation via uPAR render mice more susceptible to TTP, whereas unrestrained endogenous plasmin is able to prevent acute TTP episodes. Therefore, endogenous plasmin levels control the development of acute TTP episodes in mice and interventions to increase plasmin generation may be beneficial during acute episodes.

Disclosure of Interest: None declared.

OR154

Coversin, a novel complement C5 inhibitor and potential therapeutic agent, prevents C5 activation in patients with C5 polymorphisms

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Background: The monoclonal antibody eculizumab which prevents cleavage of complement C5 by C5 convertase, is extensively used to

treat paroxysmal nocturnal haemoglobinuria (PNH), atypical haemolytic uraemic syndrome (aHUS) and thrombotic microangiopathies. Patients with p.R885H and probably, p.R885C heterozygous C5 polymorphisms are resistant to treatment with eculizumab, causing an increased thromboembolic risk. p.R885H has an incidence of about 3.5% in Japanese, 1% in Han Chinese and is rare in other populations. Coversin, a small protein inhibitor of C5 binds to a different site on C5 than eculizumab and is a potent inhibitor of C5 activation in a wide range of mammals, we therefore hypothesised it might block C5 cleavage in patients with polymorphisms to p.R885.

Aims: To compare the ability of Coversin and eculizumab to prevent activation of human C5 in patients with C5 p.R885 polymorphisms.

Methods: Serum from six normal controls and two Caucasian patients proven (p.R885H) or believed (p.R885C), to be resistant to eculizumab, was tested using a CH50 ELISA (Quidel). Serial therapeutic concentrations of eculizumab and Coversin were used to spike patient and normal control sera *in vitro*.

Results: Eculizumab completely inhibited normal control serum at concentrations above $35 \mu\text{g mL}^{-1}$ (the expected threshold), but it only inhibited CH50 activity in serum from patients with both the p.R885 polymorphisms by 75% even at concentrations up to $100 \mu\text{g mL}^{-1}$. In contrast, Coversin inhibited complement activity equally well in resistant patients and normal controls, completely ablating activity at approximately $10 \mu\text{g mL}^{-1}$.

Conclusion: Coversin, but not eculizumab, totally inhibited complement activation in sera from two Caucasian patients with C5 polymorphisms within the same codon. Near 100% inhibition is necessary for optimal clinical control of conditions such as PNH and aHUS, so Coversin might provide a useful alternative to eculizumab in patients where no other prophylaxis option is available.

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OR155

Long-term gene therapy for thrombotic thrombocytopenic purpura using the 'Sleeping Beauty' transposon system

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Background: Congenital deficiency of ADAMTS13 may cause the life-threatening disease thrombotic thrombocytopenic purpura (TTP). The current treatment of choice is plasma infusion. However, infusion of plasma exposes patients to several risks.

Aims: To use the non-viral 'Sleeping Beauty' (SB) transposon system to obtain long-term correction of TTP in *Adamts13*^{-/-} mice triggered with recombinant human VWF (rhVWF).

Methods: Both the SB transposon and transposase (Tase)-expression plasmids were hydrodynamically injected in *Adamts13*^{-/-} mice to obtain stable murine (m) ADAMTS13 expression. Injection of the single SB transposon (no Tase) or 0.9% NaCl were used as controls. Transgene mADAMTS13 present in plasma was measured using ELISA and its proteolytic activity was assessed by analysis of the VWF multimer pattern. Mice were challenged with rhVWF to trigger TTP.

Results: Injection of both SB plasmids resulted in high, stable and long-term expression of transgene mADAMTS13 [$184 \pm 17\%$, $n = 7$, 25 weeks post injection (wpi)]. In contrast, when the single SB plasmid (no Tase) was injected, mADAMTS13 expression was initially high but transient as only low antigen levels were detected 25 wpi ($10 \pm 2\%$; $n = 7$). Interestingly, circulating high molecular weight (HMW) VWF multimers were significantly reduced in mice expressing high and stable mADAMTS13 levels ($31.7 \pm 13\%$ HMW; $n = 4$, $P < 0.001$) but not in mice with low and transient levels ($39.9 \pm 1.2\%$ HMW; $n = 5$; $P = \text{ns}$) at 25 wpi compared with *Adamts13*^{-/-} mice ($42.6 \pm 1.5\%$ HMW; $n = 5$). Moreover, at 20 wpi stable expression protected *Adamts13*^{-/-} mice from TTP as they did not develop severe thrombocytopenia ($581 \pm 35 \times 10^3 \text{ PLT } \mu\text{L}^{-1}$; $n = 7$) after challenge with rhVWF. In contrast, mice treated with only the transposon plasmid (no Tase: $216 \pm 37 \times 10^3 \text{ PLT } \mu\text{L}^{-1}$; $n = 7$) or with 0.9% NaCl ($118 \pm 10 \times 10^3 \text{ PLT } \mu\text{L}^{-1}$; $n = 6$) were not protected from TTP.

Conclusion: We successfully used the non-viral SB transposon system to realize long-term expression of active transgene mADAMTS13 in order to prevent the onset of TTP in *Adamts13*^{-/-} mice.

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Pediatric Thrombosis

OR156

Children's Hospital-Acquired Thrombosis database (CHAT): a multi-institutional database for prospective identification of independent risk factors

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Background: Pediatric hospital-acquired venous thromboembolism (HA-VTE) incidence is rising, but is low overall, requiring risk stratification to reduce unnecessary thromboprophylaxis exposure in low risk patients. Large sample sizes are needed for prospective epidemiologic studies, necessitating collaboration.

Aims: We formed the multi-institutional Children's Hospital-Acquired Thrombosis (CHAT) database to identify pertinent HA-VTE risk factors for future risk scoring.

Methods: This IRB-approved, retrospective chart review reveals HA-VTE risk factors from patients aged 0–21 years with diagnostically-validated VTE more than 48 h after hospital admission at 3 pediatric hospitals from January, 2012 to December, 2014.

Results: Three hundred and twenty-one patient records were analyzed. 57% were male, with a median age of 4 years, though most were younger than 1 year (32%) or over 12 (33%). VTE locations included upper (36%) and lower extremity (36%), jugular (9%), abdominal (6%), cerebral sinus (6%), pulmonary (6%) and cardiac (4%). 70% of sub-

jects had a central venous catheter (CVC) with anatomic/temporal relation to the VTE: PICC (62%), Femoral (25%), IJ (8%), surgically-implanted (8%), or other (8%). 61% of subjects were admitted to a pediatric, neonatal or cardiac intensive care unit. Common past medical history items were congenital heart disease (26%), cancer (19% [leukemia/lymphoma 12%, solid tumor 7%]), metabolic disease (8%), autoimmune conditions (6%), other (26%) and none (20%). Analysis of association with various acute co-morbid conditions (surgery, infection, etc.) is ongoing.

Conclusion: These risk factors reveal increased prevalence of male sex, infant or adolescent age, CVC, and ICU admission in HA-VTE cases, and co-morbidities of infection/inflammation, congenital heart disease, and cancer. Subsequent retrospective analysis will incorporate cases and controls from 4 additional institutions to obtain odds ratios that will inform a risk score and be subsequently prospectively validated in a third group of hospitals.

Disclosure of Interest: None declared.

OR157

Correlation between activated partial thromboplastin time (APTT) and anti-FXa assay in children on unfractionated heparin (UFH) therapy: an institutional experience

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Background: The American College of Chest Physicians recommends that a therapeutic APTT range in children should reflect an anti-FXa level of 0.35–0.7 IU mL⁻¹. Recent studies have shown a poor correlation between APTT and anti-FXa assays in children receiving UFH therapy.

Aims: A quality improvement (QI) initiative to retrospectively evaluate the correlation between anti-FXa and APTT drawn simultaneously in children receiving UFH at a single institution.

Methods: This QI project was deemed exempt by the Institutional Review Board. All pediatric patients (aged 0–21 years) on UFH who had both APTT and anti-FXa assays drawn simultaneously over a 4 year period (2011–2014) were included. Patient data was collected using the institution's electronic enterprise data warehouse. Anti-FXa and APTT assays were performed on the STA-R Evolution[®] Analyzer using the STA[®]-liquid Anti-FXa reagents and the STA[®]-PTT reagent, respectively. Standard statistical methods were used to summarize data (mean [±SD]). Spearman rank test (r_s) was used to assess correlation.

Results: Between 2011 and 2014, 14,207 APTTs were performed at our institution. Of these, there were 561 observations (42 distinct patients; male:female 1:1) where APTT and anti-FXa assays were drawn simultaneously. Mean patient age at the time of laboratory draw was 3.9 (±5.8) yrs. We saw an increasing trend of anti-FXa levels being drawn with APTT over the study duration (20 in 2011, 42 in 2012, 251 in 2013 and 248 in 2014). Weak correlation between APTT and anti-FXa assays was noted for the entire study cohort (r_s : 0.48). The correlation was weaker for patients < 1 year than those ≥ 1 year (r_s : 0.44 vs. 0.51) and was weakest in the neonatal intensive care unit (ICU) (r_s : 0.41) compared to the pediatric ICU (r_s : 0.53) and non-ICU patients (r_s : 0.49).

Conclusion: We confirm that APTT is not a reliable predictor of anti-FXa activity in children. Based on the above data, we are changing our institutional UFH nomogram to an anti-FXa based guideline.

Disclosure of Interest: None declared.

OR158

Age is main clinical risk factor for venous thromboembolism within a large cohort of children with acute lymphoblastic leukemia

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Background: Venous thromboembolism (VTE) is rare in children, but relatively common in children with acute lymphoblastic leukemia (ALL). Reported risk factors for VTE with ALL are asparaginase and steroid treatment. However, within the ALL population, risk factors for VTE are less clear.

Aims: To identify clinical risk factors for VTE in a large cohort of pediatric ALL patients, using a nested case-control study.

Methods: We retrospectively analyzed VTE events in 780 children with newly diagnosed precursor-B lineage or T-lineage ALL in the ages of 1–18 years, treated in the Dutch Childhood Oncology Group (DCOG) ALL-10 protocol in 6 Dutch pediatric cancer centers between October 2004 and April 2013. To identify clinical risk factors for VTE, a nested case-control study was conducted (1:2; total $n = 177$).

Results: Fifty-nine of 780 children with ALL suffered from VTE (7.6%). Events occurred during induction treatment in 35 patients (59.3%) and during medium risk intensification in 24 (40.7%). 26 of 59 children (44.1%) experienced cerebral vein thrombosis (CVT), 12 (20.3%) deep-vein thrombosis of the leg, 3 (5.1%) pulmonary embolism, and 18 (30.5%) upper limb vein or catheter-related thrombosis. A multivariate logistic regression analysis including age, sex, ALL subtype, ALL risk group and cerebral involvement of ALL showed that only age was significantly associated with VTE (OR 1.2; 95% CI 1.1–1.3 with age as continuous variable; OR 3.0; 95% CI 1.5–6.1 with age < 7 or ≥ 7 years). An expanded analysis also including baseline leukocyte and platelet count, baseline % of blasts in peripheral blood or bone marrow, sepsis, and extramedullary disease did not alter this result. In a subanalysis for CVT, age remained the only significant risk factor for VTE (OR 1.2; 95% CI 1.1–1.3 with age as continuous variable; OR 3.1; 95% CI 1.2–7.8 with age < 7 or ≥ 7 years).

Conclusion: Within this large pediatric ALL cohort, older age at diagnosis was the main risk factor for VTE and CVT, and should be considered in preventive strategies.

Disclosure of Interest: None declared.

OR159

Outcomes of lower limb deep vein thrombosis and predictors of lower limb post-thrombotic syndrome in pediatric patients

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Background: Pediatric lower limb deep vein thrombosis (LL-DVT) can lead to post-thrombotic syndrome (PTS) and other adverse outcomes. The characterization of pediatric LL-DVT is lacking.

Aims: To study the outcomes of LL-DVT in children.

Methods: Children who had ≥ one PTS assessment ≥ 6 months after DVT diagnosis were included. PTS (main outcome) was determined

by the Modified Villalta Scale. Three groups were compared: unprovoked DVT (G1), provoked DVT in neonates (G2A) and in non-neonates (G2B). Ethics approval was obtained.

Results: One hundred and sixty-five children (G1 $n = 21$ /G2A $n = 49$ /G2B $n = 95$), M:F ratio: 1.1, median age [interquartile range (IQR)]: G1 16.3 year (2.6 year), G2A 9 day (12 day), G2B 0.6 year (3.6 year) were included. The most common underlying condition and risk factors were: G2A cardiac 63%, central venous line (CVL) 86%, G2B cardiac 43%, CVL 87%. DVT treatment included: G1 anticoagulant drugs (ACD) 76%, directed lysis 24%; G2A ACD 96%; G2B ACD 98%.

Outcomes:

- Recurrent DVT: G1 19%, G2A 0%, G2B 2%, $P < .001$
- Complete DVT resolution (end of treatment): G1 24%, G2A 31%, G2B 26%, $P = .76$
- DVT extension: G1 5%, G2A 2%, G2B 8%, $P = .33$
- Pulmonary embolism: G1 29%, G2A 0%, G2B 3%, $P < .001$
- PTS: At last *f/u*, PTS was seen in G1: 75% (mild 87%, moderate 13%); G2A 33% (mild 94%, moderate 6%); G2B 54% (mild 92%, moderate 8%), $P = .01$. The highest scores seen *throughout f/u* were: G1 12, G2A 4, G2B 5

PTS prediction model: Multivariable logistic regression showed that only sex and age at DVT diagnosis were independent predictors of PTS score ≥ 2 , [odds ratio 2.4 (95% CI 1.1–5.2, $P = .03$) for males vs. females, and 1.1 (95% CI 1.1–1.2, $P < .001$) for age in yrs]. Other predictors considered in univariable analysis were: group, treatment - modality/duration/delay to start-, number of segments affected, degree of occlusion, ilio-femoral involvement, end of treatment resolution, DVT recurrence and extension.

Conclusion: Overall, CVL-related LL-DVT has more benign outcomes, especially in younger patients. Pediatric LL-PTS frequency and severity depend on patient's age and sex.

Disclosure of Interest: None declared.

OR160

Diagnosis and management of venous thromboembolism (VTE) in children: a prospective 22-year registry from a single tertiary center in Argentina

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Background: There has been a dramatic increase of VTE in children related to medical progress in the management of critically and chronically ill patients(pt). Risk factors, imaging, thrombophilia(TB), anti-thrombotic therapy(AtT) and complications have been the subject of research in the last 25 years.

Aims: To describe diagnosis and management of a pediatric prospective cohort with VTE registered during 22 years at a single tertiary center.

Methods: From May 1992 to Apr 2014, consecutive children < 18 years of age with objectively confirmed VTE were prospectively registered. Demographic and clinical data, family history of VTE, imaging, TB, AtT and outcomes were recorded. Neonates and pt with cerebral or organ specific VTE were excluded.

Results: 914pt were recruited, 525 males (57%), median age (range): 2 years (0.1–18). One or more risk factors were found in 909pt (99%), 58% also had catheters. Lower/upper/both venous systems were involved in 590pt (65%)/279pt (31%)/45pt (4%). Family history of VTE: 45pt (5%).

434pt (48%) were available for TB. Prothrombotic disorders were found in 120pt (28%): inherited disorders in 61pt (FVL:24, PT20210A:18; deficiency of PC:10, PS:7 and AT:2) and acquired disor-

ders in 59pt (AT deficiency: 29; persistently positive APA: 30). Seven pt (1.6%) had combined disorders. The AtT used was: UFH: 93pt; enoxaparin 809pt, VKA:675pt, tPA:28pt, vena cava filters, 6pt. The duration of the AtT was based on the persistence of risk factors and thrombophilia.

Recurrence or death related to VTE occurred in 11pt (1.2%) and 2pt (0.21%), respectively. Six pt (0.7%) receiving enoxaparin had major bleeding. Post-thrombotic syndrome was assessed in 325pt, 52 (16%) were severe.

Conclusion: This is the largest prospective cohort reported of pediatric VTE with long-term follow-up. Close management by specialized professionals of in and out-patients has allowed to achieve excellent results concerning short and long-term outcomes. Multicenter RCT are required to obtain stronger evidence-based recommendations on different aspects of VTE.

Disclosure of Interest: None declared.

Factor VIII – II

OR161

Hematopoietic cells a novel target for hemophilia A cell therapy

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Background: Identification of cells capable of synthesizing and releasing factor (F) VIII is critical for developing therapeutic approaches in hemophilia A (HA). Endothelial cells (EC), particularly liver sinusoidal EC, express FVIII most in the body. However, recent studies of bone marrow (BM) transplantation suggested additional cell types could synthesize and release FVIII, correcting bleeding phenotype in HA mice.

Aims: To establish the ability of murine and human hematopoietic cells (HCs) to express FVIII.

Methods: Monocytes were obtained by adhesion from peripheral blood mononuclear cells (PBMC) and then differentiated in macrophages or dendritic cells (DC) by adding M-CSF or GM-CSF and Flt3-L to medium, respectively. Megakaryocytes (MK) were differentiated from cord blood (CB) CD34 + cells through IL-6, IL-11, TPO stimulation. For xenotransplantation, $3-6 \times 10^5$ CD34+ cells were transplanted by tail vein injection in busulfan conditioned NOD-SCID- γ Null-HA mice (NSG-HA). FVIII activity was evaluated by aPTT 12 weeks after injection.

Results: By immunostaining FVIII was present in HCs isolated from PBMC, BM and CB. The identity of these cell types was verified by co-staining for FVIII and markers for monocytes, macrophages, DC and MK. Moreover, FVIII expression was verified by RT-PCR. We detected FVIII expression in mononuclear cells or EC in other organs, e.g., spleen, lungs and kidneys. Finally, we performed transplantation studies in NSG-HA mice with CD34 + cells from hCB. FACS analysis showed engraftment higher than 40% in mice up to 3 months later. aPTT performed on plasma of treated mice showed FVIII levels 2–5% of normal, sufficient to ameliorate the bleeding phenotype; indeed 8 out 12 mice survived to tail clip assay. Similar results were obtained in short term experiments (1w) injecting hCD11b+ cells in NSG-HA mice.

Conclusion: Besides EC, FVIII is expressed in HCs, offering further opportunities for understanding mechanisms in FVIII synthesis and replenishment.

Disclosure of Interest: None declared.

OR162

Relations between factor activity level and joint bleeding in a large cohort of males with mild and moderate hemophilia in the United States

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Background: The availability of longer acting treatment products raises the possibility of maintaining higher trough factor activity (FA) levels in prophylaxis to further decrease bleeding. Data are needed on target trough levels and possible differences in these targets by hemophilia type.

Aims: To assess joint bleeding according to FA level using longitudinal data collected on males with moderate or mild hemophilia receiving episodic care only.

Methods: Data were collected from annual clinic visits over a 12-year period from participants of a surveillance system established in the US HTCN. All patients or parents gave informed consent. Data on baseline FA level, age, race, health insurance, body mass index and hemophilia type (HA vs. HB) were obtained from the clinic record. The number of joint bleeds in the previous 6 months was obtained at each visit from bleed logs or patient/parent recall. Patients with inhibitors were excluded. Data from all visits were used in regression models to evaluate associations between FA and bleed rates.

Results: During the study period, data were collected on 4771 patients (3315 HA; 1456 HB) from a total of 19,979 clinic visits (mean 4.2 visits/patient). Patient age ranged from 2 to 91 years (mean 22.7, median 16 years) and baseline FA level ranged from 1% to 49% with a mean of 9.4%. The overall mean number of joint bleeds per patient was 1.2 bleeds per 6 months and was greater for patients with HA than for those with HB after adjustment for FA level (1.4 vs. 0.7 bleeds; $P < 0.001$). After adjustment for all of the studied variables, joint bleeding rates were significantly greater for patients with HA than for patients with HB. The regression models predicted 1.4 bleeds/year for HA patients and 0.6 bleeds/year for HB patients at a FA level of 15%.

Conclusion: Joint bleed rates for any given FA level were higher among HA than HB patients and target FA levels of 15% are unlikely to prevent all joint bleeding in US hemophilia patients.

Disclosure of Interest: None declared.

OR163

Characterization of interactive sites of activated coagulation factor VIII and low-density lipoprotein receptor-related protein 1

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Background: Clearance of blood coagulation factor VIII (FVIII) was shown to involve the low-density lipoprotein receptor-related protein 1 (LRP) (Saenko E. *et al*, 1999; Bovenschen N. *et al*, 2005). In LRP, the ligand-binding moiety is represented by complement-type repeats (CRs) grouped in clusters II, III and IV. In purified system, FVIII and its light chain (LCh) were found to interact with specific CRs of clusters II and IV (Meijer A. *et al*, 2007). Recently, we showed that LRP is involved in clearance of the activated form of FVIII (FVIIIa). We found that FVIIIa and its heterodimer (HDa, comprising the LCh), bind LRP cluster III, and identified the cluster's specific CRs responsible for the binding (Kurasawa J. *et al*, 2014). In present work, we further characterized the interaction of FVIIIa and LRP.

Aims: Identify all LRP CRs responsible for binding FVIIIa and the interactive site on FVIIIa.

Methods: LRP fragments were expressed in a baculovirus system and tested for binding FVIII, FVIIIa and HDa using surface plasmon resonance. Specificity of the interactions was tested by site-directed mutagenesis of the LRP fragments and in a competitive binding assay using an anti-FVIII antibody fragment, specific to FVIII LCh.

Results: LRP clusters II and IV were found to interact with FVIII, FVIIIa and HDa. As a minimal binding site of LRP for its ligands is formed by a CR doublet (Fisher C. *et al*, 2006), a set of doublets that systematically overlap clusters II and IV was generated and tested with the FVIII fragments. The interactions' specificity was confirmed in a competitive binding assay and the mutagenic study. We found that CRs 3–4 and 5–9 of cluster II, and CRs 24–26 and 28–29 of cluster IV form the binding sites for all FVIII fragments.

Conclusion: In LRP clusters II and IV, specific CRs to bind FVIIIa were identified. The interactive site of FVIIIa was found to involve its HDa. These results finalize mapping of LRP CRs responsible for the binding of FVIII and FVIIIa.

Disclosure of Interest: None declared.

OR164

AccuCopy quantification combined with pre-amplification of long-distance PCR for fast genotyping of int22 h-related inversions in haemophilia A

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Background: Int22 h-related inversions (Inv22) were found in almost one half of patients with severe haemophilia A (HA). Both LD-PCR and IS-PCR methods are used in the laboratories worldwide to detect all types of Inv22. However, a new approach for Inv22 analysis based on advanced techniques should be particularly desirable.

Aims: The aim of this study is to develop a novel method to identify all known types of Inv22 and the carrier mosaicism.

Methods: A genotyping test with two multiple LD-PCR is designed to detect Inv22. AccuCopy technique is used to quantify the products of pre-amplification of LD-PCR (AQ-PLP) with 12 or 16 cycles. A carrier mosaicism test is designed to only amplify the Inv22 allele rather than the wild-type int22 h allele. For validation of the new method for Inv22 genotyping, 50 pedigrees with severe HA previously analyzed by LD-PCR were detected by AQ-PLP.

Results: The patterns of PCR products in the genotyping test can diagnose and distinguish all known types of Inv22. AccuCopy detection is sensitive enough to represent the patterns of PLP products. The limit of detection (LOD) of AQ-PLP is 0.2% for female carrier mosaicism. Consistent results were obtained in the 50 pedigrees between the AQ-PLP and LD-PCR.

Conclusion: The AQ-PLP is confirmed to be a rapid, reliable and automatic method for identification of all known types of Inv22, and is sensitive for carrier mosaicism detection. It could be a first choice for genetic counselling in severe HA patients.

Disclosure of Interest: None declared.

OR165

***In vivo* studies of efficacy and potential immunogenicity of human factor VIII pace-furin deletion variants**

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Background: During intracellular processing B-domain deleted human factor VIII (hFVIII) is predominantly cleaved at a Paired basic Amino acid Cleaving Enzyme-Furin (PF) site (1645-RHQR-1648) to yield two polypeptide chains that are secreted as a heterodimer.

Aims: Based on studies of R1645H, we hypothesized that deletion(s) of the PF site may have higher biological activity without increased immunogenicity.

Methods: A series of hFVIII-PF deletion variants were purified ($\Delta 45$, $\Delta 1645-6(\Delta 2)$, $\Delta 1645-7(\Delta 3)$, $\Delta 1645-8(\Delta 4)$, or $\Delta 48$) or introduced into AAV8-hFVIII to assess hemostatic efficacy.

Results: *In vitro* these PF variants were secreted mostly as single chain with 2-fold higher activity than hFVIII in 2-stage aPTT. Before tail clip assay hemophilia A (HA) mice were infused with protein ($10 \mu\text{g kg}^{-1}$). Blood loss after infusion of $\Delta 3$ ($77 \mu\text{L}$) and $\Delta 4$ ($50 \mu\text{L}$) was similar to wild type mice ($68 \mu\text{L}$) and was significantly reduced compared to hFVIII ($180 \mu\text{L}$) and PBS ($393 \mu\text{L}$). Upon delivery of AAV8-hFVIII-PF in HA mice, $\Delta 45$, $\Delta 3$, and $\Delta 4$ showed a 2–4 fold increase in protein expression compared to hFVIII. To determine if these variants may break tolerance to hFVIII, we delivered the AAV8-hFVIII-PF variants to HA/phFVIII transgenic mice that express hFVIII in platelets and are tolerant to hFVIII. No anti-hFVIII IgG were detected after AAV while injection of AAV-canine FVIII (cFVIII) induced anti-cFVIII IgG. Upon challenge of these AAV-treated HA/phFVIII mice with the respective variant protein ($5 \mu\text{g}$) in Complete Freund's adjuvant (CFA) at 20 weeks post vector administration, mice injected with cFVIII had anti-cFVIII IgG ($63 \mu\text{g mL}^{-1}$) while all hFVIII-PF variants had anti-hFVIII IgG levels similar to hFVIII protein ($2 \mu\text{g mL}^{-1}$). When untreated HA/phFVIII mice were challenged with $5 \mu\text{g}$ of variant protein in CFA, none of the variants broke tolerance.

Conclusion: These data suggest that hFVIII-PF variants have enhanced hemostatic function both *in vitro* and *in vivo*, while are not more immunogenic than hFVIII. (Supported by Pfizer ASPIRE Award).

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Inflammation

OR166

Platelet factor 4 regulates B cell differentiation

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Background: Platelets are not only the cellular mediator of thrombosis, but platelets are also the most numerous blood cells with immune regulatory functions. We have shown that platelets have an important role in acquired immune development and responses, including a central role for platelets in T-helper cell homeostasis. This is in large part mediated by the chemokine platelet factor 4 (PF4). We have now found that PF4 is needed for B cell maturation in the bone marrow compartment.

Aims: Our studies demonstrate a major role for PF4 in B cell differentiation and maturation in the bone marrow.

Methods: These studies utilize wild-type (WT) and PF4 deficient (PF4^{-/-}) mice to determine the role of PF4 in B cell differentiation. Bone marrow was isolated from mice and B cell precursors enumerated by flow cytometry. B cell differentiation was examined *in vitro* using hematopoietic stem cells (HSC) isolated from mice. B cell responses were determined by ovalbumin (OVA) immunization.

Results: PF4^{-/-} mice have approximately 50% fewer B cells compared to WT mice both in the spleen and bone marrow. PF4^{-/-} mice have slightly more bone marrow HSCs and equal numbers of common lymphoid progenitor (CLP) cells compared to WT mice. However, beginning at a very early Pre-Pro-B cell stage, PF4^{-/-} mice have significantly fewer B cell progenitors. STAT5 phosphorylation (P-STAT5) is needed for early B cell differentiation and marrow B cells from PF4^{-/-} mice have less P-STAT5 that is normalized to WT status by incubation with recombinant PF4 (rPF4). Differentiation of HSC into B cells *in vitro* is reduced by PF4 blocking and increased by addition of rPF4. Finally, WT mice have increased B cell and plasma immunoglobulin responses to ovalbumin challenge compared to PF4^{-/-} mice.

Conclusion: Together our data demonstrates that PF4 increases B cell differentiation in the bone marrow compartment beginning at the early post-CLP stage by increasing STAT5 activation.

Disclosure of Interest: None declared.

OR167

Extracellular RNA induces venous thrombosis through TLR3

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Background: Vascular and circulating cells sense 'pathologic ligands' in the circulation via toll-like receptors (TLRs) and translate signals into a pro-thrombotic state possibly leading to venous thrombosis. More specifically, TLR3 recognizes self-molecules, such as extracellular RNA (eRNA), generated during tissue damage and inflammation.

Aims: We hypothesize that eRNA and TLR3 are involved in the development of venous thrombosis after vessel injury.

Methods: The ferric chloride (FeCl₃) model was used to induce thrombosis. WT or TLR3 deficient (–/–) mice received an intravenous injection of either vehicle, a specific fluorescent probe for RNA (Syto RNA Select), RNase1, poly(I:C) or RNA extracted from murine endothelial cells (eRNA) prior to thrombosis induction.

Results: Injection of RNase1 decreased the size of thrombi after FeCl₃-induced inferior vena cava injury (IVC) compared to mice treated with vehicle control. Using Syto RNA Select, we found that FeCl₃ induced RNA release and increased RNA content of thrombi of WT mice. In WT mice, but not TLR3^{-/-} mice, thrombus size was increased by eRNA or poly(I:C). Poly(I:C) treatment was associated with a recruitment of neutrophils to thrombi of WT but not TLR3^{-/-} mice. More importantly, poly(I:C) induced NET formation within thrombi of WT but not TLR3^{-/-} mice. Injection of eRNA also promoted the recruitment of neutrophils in WT but not TLR3^{-/-} mice. *In vitro*, eRNA induced the expression of CXCL5, a murine isoform of human IL-8, in WT but not TLR3^{-/-} endothelial cells. Conditioned media from HUVEC transfected with control siRNA and treated with poly(I:C) induced neutrophil migration. Transfection with a TLR3 siRNA blocked neutrophil migration. Co-incubation of conditioned media with an IL-8 receptor (CXCR2) antagonist inhibited neutrophil migration.

Conclusion: These results suggest that eRNA and TLR3 participate in thrombus formation, in part, by inducing IL-8 expression from endothelial cells leading to the recruitment of neutrophils during venous thrombosis.

Disclosure of Interest: None declared.

OR168

Circulating histones disturb intracellular calcium homeostasis and cause calcium overload to induce cardiovascular abnormalities in sepsis

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Background: Cardiac complications during severe sepsis are common and frequently lethal but the underlying mechanisms remain elusive. Recent discovery of the cytotoxicity of extracellular histones suggests that this mechanism might be relevant.

Aims: To investigate whether circulating histones contribute to sepsis-induced myocardial injury and dysfunction.

Methods: *In vitro* model of cultured cardiomyocytes. Histones intravenous infusion into mice and septic peritonitis mouse model (intraperitoneal injection of *E. coli*). A cohort of 65 severely septic patients and 27 healthy volunteers (control group).

Results: We demonstrate that histones bind to cardiomyocyte membrane to induce profound calcium influx. The consequent calcium overload activated adverse signaling pathways including the protein kinase C α -cardiac troponin I axis with detrimental effects on contraction force and rhythm. *In vivo*, injecting histones into mice caused significant increases in circulating cardiac troponins and left ventricular (LV) dysfunction with arrhythmias, including atrio-ventricular (AV) blocks and ectopic beats. In a mouse model of septic peritonitis, high circulating histone levels were observed and linearly correlated with circulating cardiac troponins ($r = 0.655$, $P < 0.001$). LV depression and arrhythmias including AV blocks were also identified. In both mouse models, cardiac complications were alleviated by anti-histone antibodies. Of clinical relevance is that in 65 patients with severe sepsis, circulating histone levels were significantly elevated and linearly correlated to cardiac troponins ($r = 0.585$, $P < 0.001$), noradrenaline doses to achieve haemodynamic stability ($r = 0.455$, $P < 0.001$) and mortality ($P = 0.008$). Furthermore, patients' sera directly induced histone-specific cardiomyocyte death *ex vivo*, which was abrogated by anti-histone antibodies.

Conclusion: These findings demonstrate a novel mechanism of sepsis-induced cardiomyopathy mediated by circulating histones which could be potentially targeted by anti-histone intervention.

Disclosure of Interest: None declared.

OR169

Platelet granule release is crucial for vascular integrity in the ischemic brain but not in the inflamed skin or lung

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Background: Recent studies have shown that platelets prevent inflammatory bleeding through (hem) ITAM-dependent mechanisms independently of aggregation and thrombus formation. While the exact mechanisms underlying this process remain unknown, it was specu-

lated that mediators released from platelet granules might play an important role.

Aims: We aimed to investigate the role of platelet granule release in maintaining vascular integrity in the course of inflammation in different organs.

Methods: Mice deficient for both Nbeal2 and Munc13-4 were generated and subjected to *in vivo* models of inflammation (inflammation of the skin/lung tissue: reverse passive Arthus reaction and LPS-triggered lung inflammation; thrombo-inflammation of the brain following ischemic stroke) and vascular integrity was assessed.

Results: Platelets from mice deficient for both Nbeal2 and Munc13-4 lack alpha-granules and are unable to secrete their dense granule content. These platelets showed impaired aggregation and adhesion to collagen under flow *ex vivo*, which translated into infinite tail bleeding times and severely defective arterial thrombus formation *in vivo*.

When subjected to *in vivo* models of skin or lung inflammation, the double mutant mice showed no signs of hemorrhage. In contrast, however, lack of platelet granule release resulted in impaired vascular integrity in the ischemic brain following transient middle cerebral artery occlusion (tMCAO) leading to increased mortality. The survivors, however, displayed reduced infarct sizes and better neurological outcomes.

Conclusion: Our data shows that deficiency in both major platelet granule types results in impaired vascular integrity in the course of thrombo-inflammatory brain infarction but not experimental inflammation of the skin or lung. This indicates that the mechanisms by which platelets maintain vascular integrity under conditions of acute inflammation differ between organs.

Disclosure of Interest: None declared.

OR170

Dual action of platelets and glycoprotein-VI in immune complex-mediated inflammation

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Background: Platelets maintain vascular integrity during inflammation. Evidence suggests that this protective function is independent of thrombus formation and involves platelet immunoreceptor tyrosine activation motif signalling. However, it remains unclear how platelets prevent inflammatory bleeding. Our hypothesis is that platelets could dampen or repair neutrophil-inflicted vascular damage.

Aims: Our objective was to determine how platelets and glycoprotein VI (GPVI) contribute to maintain vascular integrity during inflammation.

Methods: In models of immune complex (IC)-mediated inflammation combined with immunodepletion of platelets and/or neutrophils in wild-type and/or GPVI deficient mice, we investigated the contribution of platelets to the regulation of neutrophil recruitment, infiltration, and injurious activities. Also, using intravital microscopy, we analyzed the contribution of GPVI to platelet recruitment and the interactions between platelets and neutrophils at the reaction site.

Results: Depletion of neutrophils prevented skin bleeding observed in thrombocytopenic and GPVI-deficient mice subjected to dermatitis, indicating that platelets counter the deleterious effect of neutrophils. However, during dermatitis and peritonitis, neutrophil infiltration, degranulation, and oxidative stress were reduced in GPVI-deficient and thrombocytopenic mice as compared to wild-type mice. Intravital microscopy revealed that in inflamed vessels, platelets interact directly

with both neutrophils and the vascular wall. Furthermore, during IC-mediated dermatitis, intravascular binding sites for GPVI were exposed by neutrophils, and GPVI supported the recruitment of platelets to these spots.

Conclusion: Our results indicate that platelets and GPVI play a dual role in inflammation by enhancing neutrophil recruitment and damaging activities while ensuring sealing of neutrophil-inflicted vascular injury through GPVI-dependent binding of single platelets.

Disclosure of Interest: None declared.

Microparticles – I

OR171

The role of breast cancer cell microvesicles in thrombogenicity, angiogenesis and apoptosis following chemotherapy

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Background: Chemotherapy administration is associated with increased shedding of microvesicles (MVs) that are involved in thrombosis and metastasis.

Aims: Characterization of MVs obtained from breast cancer (BC) patients and from human BC cell lines prior to and post exposure to chemotherapy, and evaluation of MV effects on BC cell lines and endothelial cells (EC).

Methods: Twenty one pre-surgery and post-surgery BC patients received chemotherapy with 4 cycles of adriamycin and cyclophosphamide (AC) plus 12 weekly doses of paclitaxel. Blood samples were taken at 5 time points: before and 24 h after the 1st AC; before and 24 h after the 1st dose of paclitaxel and before the last dose of paclitaxel. MV cell origin (FACS), protein content (protein array) and thrombogenicity (FACS, FXa chromogenic assay) were analyzed. MVs were isolated from BC cell lines after exposure to chemotherapy and their thrombogenic, migration and apoptotic effects on EC and BC cell lines were assessed.

Results: The BC marker, EPCAM, was found at higher levels (7–15%) in MVs of pre-surgery patients compared to post-surgery patients (< 2%). The TF/TFPI ratio was higher (> 4) in MVs obtained prior to surgery compared to those isolated post-surgery (< 1). In both groups, the MV TF/TFPI ratio significantly increased (> 10) following chemotherapy administration. Similar results were obtained in coagulation activity assay. Patient MVs induced EC proliferation and thrombogenicity. MVs of BC- cell lines obtained after high-dose chemotherapy presented a > 5-fold increase in procoagulant activity compared to MVs isolated after low-dose chemotherapy. BC-MVs adhered to EC and penetrated into the cells. Unlike untreated cells, BC-MVs increased EC thrombogenicity and apoptosis.

Conclusion: MVs of BC patients could serve as potential markers of disease status and efficacy of therapeutic modalities. Increased thrombogenicity of MVs may indicate the need for thromboprophylaxis after chemotherapy.

Disclosure of Interest: None declared.

OR172

Circulating microparticles in deficiency of the natural anticoagulants

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Background: We have recently showed that high levels of circulating microparticles (MP) were significantly associated with increased risk of VTE in factor V Leiden (FVL) and prothrombin gene mutation (PTM) carriers, suggesting a possible contribution of MP in triggering the global prothrombotic state found in mild genetic thrombophilia.

Aims: To further elucidate the prothrombotic state linked to genetic thrombophilia, we extended this study to carriers of natural anticoagulants deficiency states [severe thrombophilia – antithrombin (AT), protein C (PC) and protein S (PS) defect].

Methods: The plasma level of annexin V-MP, endothelial-derived MP (EMP), platelet-derived MP (PMP), tissue factor-bearing MP (TF+) and the MP procoagulant activity (PPL) was measured in 132 carriers of natural anticoagulant deficiencies (25 AT, 63 PC 64 PS defects) and in 132 age and gender-matched healthy individuals.

Results: Carriers of natural anticoagulants deficiencies had higher median levels of annexin V-MP, EMP, PMP, TF+MP and PPL activity than healthy controls ($P < 0.001$, < 0.001 , < 0.01 , 0.025 and 0.03 , respectively). The carriers of AT and PC defects had significantly higher levels of annexin V-MP, EMP and PMP than controls. Carriers of PS defects had significantly higher levels of annexin V-MP, EMP and TF+MP than controls. The carriers with high levels of annexin V-MP, EMP and PMP had an adjusted OR for VTE of 3.36 (95% CI, 1.59–7.11), 9.26 (95% CI, 3.55–24.1) and 2.72 (95% CI, 1.16–6.38), respectively.

Conclusion: We showed higher levels of MP in AT, PC and PS defects compared to controls. We confirm a role of circulating MP in the development of VTE in severe hereditary thrombophilia, possibly by a mechanism of endothelial dysfunction (the highest OR for VTE was seen in EMP). Further studies are needed to better define the role of MP as triggering factors for the thrombotic complications characterizing hereditary thrombophilic defects.

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OR173

Cellular microparticles as predictive markers for thrombosis and other clinical events in patients with implanted LVADs

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Background: Current methods that identify left ventricular assist device (LVAD)-associated adverse events have low sensitivity hindering early prediction and timely clinical intervention. Microparticles (MP), formed upon cellular activation or exposure to altered shear stress, may mediate hemostatic or inflammatory alterations.

Aims: To determine whether changes in blood MP levels are observed prior to the occurrence of adverse events in LVAD patients.

Methods: Blood samples collected peri-operatively and during post-op clinic visits from patients implanted with a HeartMate II LVAD (Thoratec) were centrifuged to platelet poor plasma and then ultracentrifuged to pellet the MP. MP rich samples were stained with a

membrane dye and either: CD41 (platelets), CD45 (WBC), CD146 (endothelium) or CD235 (RBC) then analyzed by flow cytometry. Six healthy individuals served to establish normal MP levels.

Results: From 21 patients studied, thrombosis (confirmed or suspected based on elevated LDH) occurred 9 times in 7 patients (mean 155 ± 111 days post-implant; range 34–350 days). Months prior to the diagnosis of thrombus, elevated CD41 + (2–6 SD above normal), CD45+ (3–5 SD) and CD146+ (5–8 SD) MP were observed. CD41+ (3–6 SD), CD45+ (2–5 SD), CD146+ (1–8 SD) and CD235+ (2–5 SD) MP were increased 2 months prior to LDH elevation. Elevations > 6 SD above normal in all MP were noted in one patient 3 days prior to hemolysis, but were not observed in a separate patient's sample collected 43 days prior to hemolysis. All patients had MP within 1 SD of normal for the first 2 months following implant; patients without events remained within or near normal range.

Conclusion: Quantitation of MP may be useful to identify thrombosis in patients with implanted LVADs in a timely manner (hemolysis and bleeding events may not be as clearly associated with sustained elevations of MP) allowing for early intervention and correction. Further evaluation of MP in a larger population is warranted.

Disclosure of Interest: None declared.

OR174

Analysis of exosome-like membrane microparticles released from TRAP-activated platelets

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Background: Elevated counts of platelet membrane microparticles (PMPs) in circulation are associated with various diseases. PMPs are also released from platelets (PLTs) during processing and storage of PLT transfusion products.

Aims: To characterize different populations of PMPs released from TRAP-activated PLTs.

Methods: Flow cytometry (FC), nanoparticle tracking analysis (NTA), confocal microscopy (CM), electron microscopy (SEM and TEM), and atomic force microscopy (AFM) was used. Proteome and lipid composition were analyzed by mass spectrometry (MS) and thrombin generation (TG) activity of PMPs was assayed.

Results: CM showed that PMPs were formed in TRAP-activated PLTs from both plasma as well as internal membranes; the mitotracker labeling detected mitochondria within PMP populations. NTA showed that PMP sizes range from 80 nm to 700 nm. The larger PMPs (PMV20) were sedimented at $20,000 \times g$ followed by $100,000 \times g$ centrifugation for isolation of exosome-like PMPs (PMV100); PMP20 and PMP100 contained 61% and 94% of PMPs < 300 nm, respectively. Phosphatidylethanolamine (PE) and phosphatidylserine (PS) content in PMP100 was approximately twice higher than in PMP20; the cholesterol ester (CE)/phospholipid ratio was 2.7 times higher in PMP100 compared to PMP20. Proteome analysis revealed the presence of PLT marker CD41 and exosome markers CD9 and Hsp70 in both PMP20 and PMP100. Both PMP populations were pro-coagulant *in vitro*; TG activity (thrombin peak height 10^{-9} PMPs) was 1.8 times higher for PMP20 compared to PMP100.

Conclusion: Activated PLTs release heterogeneous populations of PMPs differing in size, protein and phospholipid composition. TG activity of PMPs was observed, including small exosome-like PMP population, rich in PS. Analysis of exosome-like PMPs requires high resolution methods, such as NTA, AFM, and TEM. These findings and conclusions have not been formally disseminated by FDA and should not be construed to represent any Agency determination or policy.

Disclosure of Interest: None declared.

OR175

Peptidyl-prolyl isomerase 1 (PIN1) preserves the phosphorylation state of tissue factor and prolongs the release of TF within microvesicles

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Background: Control of the surface activity and release of tissue factor (TF) is critical for correct haemostasis. The exposure and release of TF is regulated by post-translational modifications of its cytoplasmic domain and particularly the phosphorylation of serine residues 253 and 258. Double-phosphorylation of TF generates a possible binding site for the enzyme peptidyl-prolyl isomerase 1 (Pin1).

Aims: To examine the potential of Pin1 to interact with the cytoplasmic domain of TF and explore the outcome on TF function.

Methods: MDA-MB-231 cells were incubated with Pin1 inhibitor Juglone, or its analogue control Plumbagin. Furthermore, Pin1 expression was suppressed using specific siRNA to Pin1. TF release into microvesicles as well as the phosphorylation state of cellular TF was assessed following cell activation. Finally, the ability of an HRP-conjugated recombinant Pin1 to bind to peptides of the C-terminus of TF, synthesised in non-phosphorylated, single-phosphorylated and double-phosphorylated forms was examined by binding assays, as well as spectroscopically using pNA-labelled peptides, and the relative dissociation constants (Kd) determined.

Results: Inhibition of Pin1 using Juglone, or siRNA-mediated suppression of Pin1 expression resulted in the early dephosphorylation of TF at serine 253 and premature termination of TF release as microvesicles compared to cells pre-incubated with Plumbagin, cells transfected with control siRNA or untreated cells. Recombinant Pin1 was able to interact with both 258-phosphorylated and double-phosphorylated TF peptides with high affinities, while exhibiting lowest affinity for the 253-phosphorylated peptide.

Conclusion: Pin1 is a fast-acting enzyme which is utilised by cells to protect the phosphorylation state of TF in activated cells. This in turn prevents the premature termination of TF activity and release and therefore regulates blood coagulation ensuring adequate haemostasis.

Disclosure of Interest: None declared.

Rare Platelet Disorders

OR176

Diagnosis of inherited bleeding, thrombotic and platelet diseases by next generation sequencing

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Background: Most monogenic inherited bleeding, thrombotic and platelet disorders (BTPD) except Haemophilia and von Willebrand's diseases are extremely rare. Reaching a conclusive laboratory diagnosis remains challenging in the routine health care settings. Next generation sequencing (NGS) will change their laboratory diagnosis bringing benefits to patient care.

Aims: We aim to deliver an NGS platform and a robust bioinformatics analysis pipeline for cheaper, faster and more accurate diagnosis for the above group of patients. To achieve good sensitivity and specificity we continue to develop and maintain a stable and sustainable freely accessible database with pertinent findings to support the generation of clinic reports based on NGS results.

Methods: An NGS platform was developed to pull down exons, UTRs and the 1500 bp upstream region of nearly 100 genes known to be

causative of BTPD. Gene-specific knowledge was provided by experts and combined with accurate information about transcripts in blood cell progenitors and hepatocytes, and germline variant of ~80,000 control DNA samples. This annotation supports the automatic generation of genotype reports rich in clinically relevant information.

Results: Genes and transcripts in the capture array were annotated and submitted to the publicly accessible Locus Reference Genomic database (<http://www.lrg-sequence.org/>). DNA samples from patients with BTPDs of known ($n = 178$) and unknown ($n = 122$) molecular aetiology were sequenced resulting in 99.2% coverage of the 1 Mb target region at 50× fold or higher. Variants were confirmed in all samples with known mutations.

Conclusion: An NGS platform is now available for the genetic diagnosis of BTPDs. The accuracy of assigning pathogenicity scores to rare variants in BTPD genes will greatly improve because of the continued increase in the number of publicly accessible genome sequencing files from control individuals.

Disclosure of Interest: None declared.

OR177

Antithrombin reduction improves coagulation in rare bleeding disorder plasma

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Background: We are currently investigating the use of RNA interference (RNAi) to target the natural anticoagulant antithrombin (AT) as strategy to improve thrombin generation, and therefore hemostasis, in hemophilia. ALN-AT3, a subcutaneously administered investigational RNAi therapeutic targeting AT, is currently being evaluated in persons with hemophilia in a Phase 1 study (NCT02035605). Based on its mechanism of action, AT reduction could potentially offer benefit in other rare bleeding disorders (RBDs) arising from insufficient thrombin generation.

Aims: RBDs associated with deficiencies in procoagulant factors (e.g. FV, FVII, and FXI) result in abnormal profiles in various coagulation assays, such as thrombin generation, prothrombin time (PT), and activated partial thromboplastin time (aPTT). The aim of the work was to explore the effect of reducing AT in different RBD settings, modeled using plasma samples with reduced levels of different individual procoagulant factors.

Methods: Human plasma samples deficient in specific factors (FV, FVII, or FXI) were immunodepleted of AT. The coagulation profile of these plasma samples was analyzed by thrombin generation (calibrated automated thrombogram [CAT] method), PT and aPTT.

Results: AT reduction led to an increase in thrombin generation and normalization of coagulation parameters for FV, FVII and FXI deficient plasma samples. CAT measurements showed an increase in peak thrombin and endogenous thrombin potential (ETP) after AT depletion for FV, FVII and FXI deficient plasma samples. Moreover, AT depletion also decreased the prolonged PT for FVII deficient plasma and decreased the prolonged aPTT for FXI deficient plasma.

Conclusion: These data suggest that AT reduction may offer potential benefit in RBDs, in particular those associated with FV, FVII, or FXI deficiencies.

Disclosure of Interest: A. Sehgal Employee of: Alnylam Pharmaceuticals, K. Qian Employee of: Alnylam Pharmaceuticals, J. Hettinger Employee of: Alnylam Pharmaceuticals, B. Sorensen Employee of: Alnylam Pharmaceuticals, A. Akinc Employee of: Alnylam Pharmaceuticals.

OR178

Long-term correction of canine FVII deficiency using AAV-mediated expression of zymogen FVII

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Background: Factor VII deficiency (FVII) is a bleeding disorder that is currently treated by protein replacement. Based on clinical data, a mild elevation of plasma FVII levels (15% normal, 75 ng mL⁻¹) results in mostly asymptomatic disease, suggesting that FVII deficiency is a good target for gene therapy.

Aims: To study the efficacy and safety of continuous expression of canine FVII (cFVII) mediated by Adeno-associated viral (AAV) vector delivery in dogs with < 4% FVII activity, the only available large animal model.

Methods: Dogs received an AAV serotype 8 expressing cFVII from a liver specific promoter at escalating doses (vector genomes kg⁻¹): high (4.95E13, HD), middle (2E12, MD), low (6E11, LD) and very low (2E11, VLD). Efficacy was measured using an antigen ELISA and a prothrombin time (PT) clotting assay sensitive to FVII. Safety was monitored with hemostatic, biochemical and overall animal physiology parameters.

Results: We confirmed that dogs with < 4% FVII activity have < 4% FVII antigen [normal: 1040 ± 380 ng mL⁻¹], thus representing the majority of human patients. After AAV delivery in these dogs, we observed a PT reduction to normal or near normal levels as a result of cFVII transgene expression, in a vector dose-dependent fashion. At the expression plateau, cFVII antigen levels were 7280 ± 1800, 320 ± 68, 170 ± 62 and 17 ± 16 ng mL⁻¹ (HD to VLD). This corresponded to 800%, 30%, 16% and 2% of normal (HD to VLD) and has been stable for over 2.6, 1.7, 1.3 and 0.8 years (ongoing, HD to VLD). Complete blood counts, serum chemistries, D-dimer and fibrinogen levels remained normal, indicating the lack of hemostatic or organ toxicity, even for the highest expressing dog.

Conclusion: For the first time in an animal model representing the majority of human patients, we demonstrate that AAV-mediated FVII gene transfer is efficacious, safe and long-term with a very wide window of cFVII expression. Importantly, a dose of 6E11 vg kg⁻¹ (considered immunologically safe in humans) results in clinically therapeutic expression.

Disclosure of Interest: None declared.

OR179

Engineered transcription factors (TALE-TF) as potential therapeutic strategy for coagulation factor deficiencies caused by promoter mutations

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Background: Transcription impairment by mutations represents a small but considerable cause of severe coagulation factor defects and of other genetic diseases. Transcription activator-like effectors fused with transcriptional activator domains (TALE-TFs) targeting promoter regions have shown their efficacy in enhancing gene expression.

Aims: To exploit engineered TALE-TFs for the rescue of coagulation factor gene expression impaired by promoter mutations. As model we chose two *F7* promoter mutations (−94C>G; −61T>G) causing severe factor VII deficiency.

Methods: We created four plasmids expressing TALE-TF (TF1-4) targeting SP1/HNF4 *F7* promoter regions. Reporter gene assays were conducted by using plasmids expressing luciferase under the control of FVII promoter variants (wt, -94G and -61G) in HepG2 cells. HepG2 and Hek293 were transfected with TALE-TFs alone to evaluate FVII endogenous expression through RT-PCR and qPCR analyses.

Results: Transcriptional activity in reporter gene assays was abolished by the -94G and -61G changes, thus confirming their causative effect. Co-transfection with the different TALE-TFs demonstrated that TF4, targeting a sequence between the mutations, induced a robust increase of gene expression (20–50 fold) for both *F7* mutants. Besides, TF4 had no effect on transcription of an unrelated (*Slug*) promoter, and its activity was abolished by three changes in the *F7* promoter target sequence, thus supporting TF4 specificity. Moreover, TF4 appreciably increased the endogenous *F7* transcription and mRNA levels in HepG2 cells (2.3 ± 0.2 fold), and induced *F7* expression in Hek293 cells that do not virtually express FVII.

Conclusion: These data with reporter genes and in the chromatin context, provide the first proof-of-principle that a unique appropriately designed TALE-TF restores gene expression in the presence of two severe promoter mutations, which might lay the foundation for the development of an innovative therapeutic approach for coagulation disorders as well as other genetic disorders.

Disclosure of Interest: None declared.

OR180

Studies of MCFD2 knockout mice suggest alternative pathways for FV, FVIII and alpha-1-antitrypsin secretion

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Background: Mutations in LMAN1 and MCFD2 cause a bleeding disorder called combined deficiency of factor V (FV) and factor VIII (FVIII). LMAN1 is a transmembrane lectin that forms a stable complex with MCFD2. The LMAN1-MCFD2 complex is a cargo receptor for efficient ER-to-Golgi transport of FV and FVIII. However, FV and FVIII are still present (5–30% of normal) in patients with either LMAN1 or MCFD2 mutations, indicating reduced secretion. No humans with both LMAN1 and MCFD2 mutations have been reported.

Aims: We aim to investigate whether the LMAN1-MCFD2 pathway is solely responsible for FV/FVIII secretion.

Methods: We generated a MCFD2-knockout (KO) mouse from a vector designed to delete both exons 2 and 3 from the 4-exon *Mcf2* gene, and characterized phenotypes of both single and LMAN1/MCFD2 double knockout (DKO) mice.

Results: MCFD2 and DKO mice are viable and fertile. FV and FVIII levels are lower in MCFD2 KO mice compared to the previously reported LMAN1 KO mice, consistent with the human observations and suggesting a more direct role of MCFD2 in cargo receptor function. Surprisingly, FV and FVIII in DKO mice match the higher levels found in LMAN1 KO mice, not the lower levels found in MCFD2 mice. This observation could be explained by a compensatory increase in an alternative FV/FVIII secretion pathway due to complete ablation of the LMAN1-MCFD2 pathway in DKO mice. RNA-seq studies suggest candidate proteins in such pathways. In addition, we also observed alpha-1-antitrypsin (AAT) accumulation in the ER of liver hepatocytes in both single KOs and DKO mice *in vivo*, suggesting that both LMAN1 and MCFD2 are involved in the ER-Golgi transport of AAT. However, intracellular accumulation of AAT is not accompanied by a decrease in plasma AAT level, suggesting that plasma AAT is subject to additional levels of control.

Conclusion: Taken together, our results suggest that, in addition to the LMAN1-MCFD2 pathway, alternative secretion pathways exist for FV/FVIII and AAT.

Disclosure of Interest: None declared.

Platelets – Signal Transduction I

OR181

A novel knock-in mouse reveals an essential role for the CLEC-2 hemITAM in development

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Background: The hemITAM containing receptor C-type lectin-like receptor 2 (CLEC-2), which signals as homodimer, is important for platelet function in development and haemostasis. CLEC-2 knockout mice show embryonic bleeding and defective lymph development. Loss of CLEC-2 also causes destabilisation of haemostasis and impaired thrombosis. The mechanisms behind these phenotypes are incompletely understood.

Aims: To investigate the requirement of CLEC-2 signalling in haemostasis and development.

Methods: We have generated a knock-in mouse (CLEC-2^{K1}) in which the critical tyrosine in the CLEC-2 hemITAM is replaced by an alanine. This mutation was previously demonstrated to abolish the signalling of CLEC-2 *in vitro* without affecting receptor surface expression. This is the first approach to specifically target the hemITAM of CLEC-2 *in vivo*.

Results: CLEC-2^{K1/K1} embryos show bleeding, blood-filled lymphatics and die perinatally, phenocopying CLEC-2 knockout mice. CLEC-2^{K1/+} mice appear healthy; have normal platelet counts, platelet size and unaltered tail bleeding times. Despite normal CLEC-2 levels CLEC-2^{K1/+} platelets show abolished responses to a CLEC-2 activating antibody and rhodocytin in aggregometry and flow cytometry which can be overcome by secondary antibody crosslinking and increased agonist concentrations respectively.

Conclusion: As predicted by *in vitro* experiments, loss of the hemITAM prevents CLEC-2 signalling. We could confirm that CLEC-2 signalling is required for lymph-vessel separation. The normal lymphatics in CLEC-2^{K1/+} mice suggest either that 25% of signal-competent CLEC-2 dimers are sufficient for this process, or that clustering of CLEC-2 occurs during lymph-vessel separation which overcomes the dominant negative effect of the hemITAM mutation as seen *in vitro*. Work is continuing to assess the role of CLEC-2 in haemostasis in these mice.

Disclosure of Interest: None declared. This work was funded by the Deutsche Forschungsgemeinschaft (SFB 688).

OR182

Apolipoprotein A-I enhances protease-activated receptor 1-dependent cytoprotective signaling by activated protein C

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Background: Activated protein C (APC) signaling via protease-activated receptor 1 (PAR1) is vasculoprotective, anti-inflammatory and of therapeutic benefit in models of inflammatory disease. However, PAR1 activation by APC is extremely inefficient and only occurs *in vitro* at supra-physiological APC concentrations. Consequently, we

hypothesized unidentified plasma-based cofactors exist that potentiate cytoprotective PAR1 signaling by APC.

Aims: To identify novel cofactors that enhance PAR1 proteolysis by APC.

Methods: We assessed PAR1 proteolysis and signaling using assays of endothelial cell (EC) barrier integrity and PAR1 reporter proteolysis on transfected HEK293T cells.

Results: PAR1-dependent EC barrier protection by APC from thrombin-induced leakage was dramatically enhanced by the presence of apolipoprotein A-I (Apo A-I), the major protein constituent of high-density lipoprotein. Apo A-I enabled half-maximal maintenance of EC barrier integrity at 30-fold lower APC concentration (250 pM) than when Apo A-I was absent. Moreover, Apo A-I dose-dependently increased PAR1 proteolysis on PAR1 reporter transfected cells. Notably, Apo A-I obviated the normal requirement for endothelial protein C receptor (EPCR) binding by APC for PAR1 signaling, enabling EC barrier protection by APC in the presence of anti-EPCR antibodies and with an APC mutant with no EPCR affinity (APC^{L8F}). In contrast, EC barrier protection by APC in the presence of Apo A-I was blocked by a PAR1 antagonist and did not occur in the presence of an APC mutant unable to cleave PAR1 (APC^{E330A}), indicating Apo A-I-enhanced APC signaling remained wholly PAR1-dependent. Remarkably, combination of Apo A-I with the APC- β glycoform (APC^{N329Q}) invoked half-maximal EC barrier protection at 90-fold less APC (80 pM) than when wild type APC was used alone.

Conclusion: This study identifies Apo A-I as a novel endogenous mediator of PAR1 activation by APC and highlights molecular elements that enable APC cytoprotective signaling with thrombin-like potency.

Disclosure of Interest: None declared.

OR183

Regulation of integrin α IIb β 3-mediated platelet spreading by the receptor-like tyrosine phosphatases PTPRA and PTPRE

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Background: Platelet adhesion and thrombus formation at sites of vascular injury is dependent on bi-directional signaling by the integrin α IIb β 3. The structurally-related receptor-like protein-tyrosine phosphatases (PTPs) PTPRA and PTPRE are established regulators of Src family kinases (SFKs) downstream of integrins in other cell types; however, their functions in platelets have yet to be determined.

Aims: The aim of this study was to determine the functions of PTPRA and PTPRE in regulating α IIb β 3-dependent platelet function and signaling.

Methods: Platelets from PTPRA and PTPRE single and double knock-out (KO) mouse models were analysed for functional and signaling defects using standard assays.

Results: PTPRA and PTPRE were both detected in human and mouse platelets by western blotting. Platelet counts and volumes were normal in PTPRA, PTPRE and double KO mice. PTPRA-deficient platelets aggregated normally in response to low and high concentrations of the GPVI-specific agonist collagen-related peptide (CRP), anti-CLEC-2

antibody and thrombin. PTPRE-deficient platelets aggregated normally in response to all agonists tested, except anti-CLEC-2 antibody, exhibiting a minor delay in aggregation that was exacerbated in PTPRA/E double-deficient platelets. Tyrosine phosphorylation was marginally reduced at early time points following CRP-stimulation in PTPRE and double KO platelets, which was corrected at later time points. Platelets from all three KO mouse models exhibited marked reductions in filopodia and lamellipodia formation on a fibrinogen-coated surface, suggesting attenuated outside-in α IIb β 3 signaling.

Conclusion: Findings from this study demonstrate that PTPRA and PTPRE play specialized roles in regulating α IIb β 3-mediated platelet spreading on fibrinogen, most likely through the regulation of SFK activity. Future studies will investigate the ability of PTPRA/E-deficient platelets to form stable thrombi under pathological conditions.

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Disclosure of Interest: None declared.

OR184

G protein-coupled receptor kinase 6 regulates platelet activation and thrombus formation through selective GPCR desensitization

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Background: Many platelet agonists mediate their cellular effects through GPCRs to induce platelet activation. Considering the crucial roles of GPCR kinases (GRKs) in GPCR functions, little is known regarding the regulation of GPCR signaling by GRKs in platelets.

Aims: To identify the molecular basis for regulation of specific GPCR desensitization by GRK6 in platelets.

Methods: We used mice lacking GRK6 to evaluate the functional role of GRK6 in platelet activation.

Results: Platelet aggregation, dense- and α -granule secretion, and fibrinogen receptor activation induced by ADP, U46619, thrombin, and AYPGKF were significantly potentiated in GRK6 $-/-$ platelets compared to WT platelets. However, CRP-induced platelet aggregation and secretion were not affected in GRK6 $-/-$ platelets. Interestingly, platelet aggregation induced by co-stimulation of serotonin and epinephrine which activate Gq-coupled 5HT_{2A} and Gz-coupled α_{2A} adrenergic receptor, respectively, was not affected in GRK6 $-/-$ platelets, suggesting GRK6 is involved in specific GPCR regulation. In addition, platelet aggregation was restored in response to second challenge of ADP, U46619, thrombin, and AYPGKF in GRK6 $-/-$ platelets whereas re-stimulation of agonist failed to induce aggregation in WT platelets, indicating GRK6 contributes to P2Y₁, P2Y₁₂, thromboxane A₂, and PAR receptor desensitization. The surface expression level of PAR4 upon AYPGKF stimulation in GRK6 $-/-$ platelets was higher than WT platelets, indicating a crucial role of GRK6 in internalization of activated PAR4 receptor. Furthermore, AYPGKF-induced Akt, ERK, and PKC δ phosphorylations were significantly potentiated and sustained in GRK6 $-/-$ platelets. Finally, GRK6 $-/-$ mice show an enhanced and stable thrombus formation after FeCl₃ injury to the carotid artery, indicating GRK6 is critical for thrombus formation *in vivo*.

Conclusion: GRK6 plays an important role in regulating platelet functional responses through selective GPCR desensitization.

Disclosure of Interest: None declared.

OR185

Procoagulant platelets are undergoing cyclophilin D mediated necrosis with differential involvement in occlusive vs. non-occlusive models of thrombosis

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Background: A subset of platelets fulfils a procoagulant role in haemostasis and thrombosis, enabling thrombin burst required for fibrin formation & clot stability at sites of vascular injury. Excess procoagulant activity is linked with pathological thrombosis. The identity of procoagulant platelets is controversial & study of its functional role hampered by lack of specific & sensitive markers.

Aims: Use synthetic dithiol alkylator GSAO to image & explore the role of procoagulant platelets.

Methods: FACS, Co-IP, Calibrated Automated Thrombogram, Confocal intravital microscopy.

Results: GSAO specifically & rapidly enters a subset of agonist-stimulated, cyclophilin D-dependent necrotic platelets & is retained in cytoplasm via covalent reaction with thromboxane A synthase-1. This subset is measurable by FACS & visualised by fluorescent microscopy. GSAO+ platelets are generated *ex vivo* within seconds of agonist stimulation; membrane compromise coincident with mitochondrial depolarisation. GSAO+ platelets are phenotypically procoagulant shown by surface colocalisation with FXa & phosphatidylserine, & functionally procoagulant with capacity to support thrombin generation *in vitro*. Real time intravital confocal microscopy show GSAO+ platelets in occlusive platelet thrombi after 8% FeCl₃ stimulation, few GSAO+ platelets seen in non-occlusive thrombi after 6% FeCl₃ or laser injury murine models of thrombosis. GSAO+ platelets are spatially associated with sites of fibrin formation, demonstrating its procoagulant role *in vivo*. Aspirin did not affect procoagulant platelet function via global thrombin generation assays. Cyclophilin D blockade reduced procoagulant function & addition of aspirin enhanced this reduction.

Conclusion: Necrotic platelets are functionally procoagulant *in vitro*, & generated after strong thrombotic stimuli *in vivo*, providing the surface for fibrin formation. Procoagulant platelets can be targeted via cyclophilin D. GSAO can be a probe used to study the role of platelet necrosis in haemostasis & thrombosis.

Disclosure of Interest: None declared.

Antiphospholipid antibodies – II

OR186

Activated protein C resistance determined by thrombin generation is predictive of thrombosis in antiphospholipid-positive patients and associated auto-immune diseases: a multicenter prospective cohort study

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Background: Activated protein C resistance (APCr) has been described in antiphospholipid antibodies (aPL) positive patients, however it is uncertain whether it can identify high risk patient for thrombosis.

Aims: To investigate the validity of APCr determined by Thrombin Generation (TG) to predict thrombosis in patients with autoimmune diseases.

Methods: This multicenter prospective cohort study included consecutive patients with aPL or Systemic Lupus Erythematosus. Blood was drawn at baseline for aPL assays and Platelet-Rich Plasma was prepared for TG using Calibrated Automated Thrombography. TG curve was recorded in absence and presence of APC to assess the overall response to APC using APC concentration needed to decrease by 50% the initial area under the TG curve (IC₅₀-APC). APCr was defined by an IC₅₀-APC above the 3rd quartile of patients. A Cox proportional hazards model assessed the validity of APCr and identified other potential independent predictors of thrombosis.

Results: One hundred and thirty-seven patients (mean age: 43.5 ± 15.4 year-old; 107 women) were followed for a mean duration of 43.1 ± 20.7 months (491.5 patient-years). Sixty one had a history of one or several thrombotic manifestations. During follow-up, 16 patients experienced a thrombotic event. In univariate analysis, APCr was significantly associated with an increased risk for thrombosis during follow-up [HR = 3.14 (95% CI: 1.13–8.76)]. Furthermore, IC₅₀-APC values were highly correlated with titers of both IgG antibodies directed against domain I of β₂-glycoprotein I and IgG anti-phosphatidylserine/prothrombin antibodies. In multivariate analysis, when hypertension and history of arterial thrombosis were added to the model, APCr remained a significant predictor of thrombosis [HR = 3.0 (CI 95%; 1.05–8.54)].

Conclusion: This study demonstrates for the first time that APCr determined by TG is a significant predictor of thrombosis in patients with aPL and associated auto-immune diseases.

Disclosure of Interest: None declared.

OR187

Effects of new (direct) oral anticoagulants on lupus anticoagulant assays

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Background: Laboratory diagnosis of antiphospholipid syndrome (APS) is based on prolongation of at least one coagulation assay [diluted Russell's viper venom (dRVVT) or activated partial thromboplastin time (APTT)] which normalizes after addition of phospholipids – lupus anticoagulant (LA). New oral anticoagulants [NOAC – dabigatran (DAB), rivaroxaban (RIV) and apixaban (API)] may be used in the treatment of APS and may influence LA testing.

Aims: We have tested the effects of NOAC on LA in patients treated with these drugs in real life settings.

Methods: Plasmas from patients with atrial fibrillation treated with DAB ($n = 30$), RIV ($n = 20$) and API ($n = 17$) and known not to have LA were tested with dRVVT (LA screen and LA confirm Life Diagn) and APTT (PTT LA Diagn. Stago and Actin FS Siemens Healthcare Diagn). The ratio < 1.2 was considered negative, > 1.4 positive, while if 1.2 – 1.4 LA could not be ruled out. Plasma conc. were DAB: 8 – $172 \mu\text{g L}^{-1}$, RIV: 8 – $437 \mu\text{g L}^{-1}$ and API: 36 – $178 \mu\text{g L}^{-1}$.

Results: Eight out of 30 samples with DAB were negative, 4 plasmas were LA positive. In all but 2 samples confirmation tests were also prolonged. DAB conc. correlated with dRVVT ($r = 0.75$ $P < 0.0001$) and APTT ($r = 0.55$, $P < 0.005$) assays, while ratio > 1.2 was observed even with the lowest conc. Negative LA was only in 1 sample with RIV (conc. $8 \mu\text{g L}^{-1}$), while 50% of samples were LA-positive. Confirmation tests were prolonged with RIV conc. $> 100 \mu\text{g L}^{-1}$. RIV conc. correlated with dRVVT assays ($r = 0.86$ $P < 0.0001$) and dRVVT and APTT ratio ($r = 0.56$ $P = 0.01$). LA was positive in 7 (41%) samples with API. Correlation between API conc. and dRVVT confirm test was observed ($r = 0.83$, $P < 0.001$) and a conc. $> 100 \mu\text{g L}^{-1}$ was associated with prolongation of confirm assay.

Conclusion: The risk of overestimation of LA detection is present in samples from patients treated with NOAC, particularly RIV. Therefore LA testing should not be performed during the treatment with NOAC. Prolongation of confirmation assays may be helpful for the recognition of false positivity, especially in the case of DAB.

Disclosure of Interest: None declared.

OR188

Obstetric antiphospholipid syndrome: early LMWH-induced variations of angiogenic factors predict placenta-mediated pregnancy complications

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Background: Late pregnancy complications are still frequent in treated pregnant women with the obstetric antiphospholipid syndrome

(oAPS). LMWH can mobilise angiogenic factors, among which placental growth factor (PGF) and the inhibitor soluble VEGF receptor (sFlt1). Antiphospholipid antibodies (aPLabs) can impair the endometrial angiogenesis and trophoblastic functions through the modulation of angiogenic factors.

Aims: To study the LMWH-induced early variations of angiogenic factors and their prognostic value in newly pregnant oAPS women.

Methods: The study was performed in the 513 oAPS women included into the NOH-APS cohort, who began a new pregnancy treated with enoxaparin 40 mg + aspirin 100 mg daily (Blood 2014; 123: 404–13). Frozen plasma aliquotes from blood sampled on EDTA, obtained just before and 4 days after the beginning of LMWH injections (platelet count checks), were tested for PGF and sFlt1 concentrations (ELISA, R&D Systems, Abingdon, UK). Clinical outcomes were the occurrence of placenta mediated complications (PMC: preeclampsia, placental abruption, small for gestational age neonate) and of PMC + stillbirths (PMC-Still). Multivariate logistic regression analysis models and ROC curves were computed.

Results: Enoxaparin induced significant and correlated PGF and sFlt1 increases. Variations in PGF were lower, and variations in sFlt1 higher, in women who developed PMC or PMC-Still. The ratio between variations of PGF and of sFlt1 were lower in case of complications, with a dose-effect relationship between quartiles and clinical risk. This ratio remains an independent risk factor after adjustment for all covariates (pregnancy loss type, aPLab triple positivity,...). The AUC under the ROC curve is 0.74 (0.68–0.80) for PMC and 0.73 (0.68–0.79) for PMC-Still.

Conclusion: In newly pregnant women with oAPS, the early LMWH-induced mobilisation of angiogenic factors can indicate the clinical risk of late complications. The imbalance in angiogenic factors might be a clinically-relevant therapeutic target.

Disclosure of Interest: None declared.

OR189

Increased risk for pulmonary hypertension associated with antiphospholipid antibodies in patients with systemic lupus erythematosus: a meta-analysis of echocardiographic studies

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Background: Pulmonary hypertension (PH) is present in patients with systemic lupus erythematosus (SLE), and the role of antiphospholipid antibodies (aPL) is controversial.

Aims: To estimate the risk of PH, including pulmonary arterial hypertension (PAH) (i.e. without history of pulmonary embolism), associated with aPL in patients with SLE.

Methods: Studies were selected if they investigated the association between aPL and PH in SLE patients and if aPL-negative patients were included for comparison. Data sources were Pubmed, Embase, Cochrane Library, hand search, congress abstracts, and reference lists of studies, without language restrictions. Risk estimates were independently extracted by 2 investigators. Pooled effect estimates were obtained by using the Mantel-Haenszel method (random effects).

Results: Of 734 identified abstracts, 36 primary studies (5 cohorts, 15 case-control, 16 cross-sectional) met inclusion criteria, including 4190 SLE patients and 478 cases of PH. Prevalence of PH in aPL-positive vs. aPL-negative SLE patients was 16% ($n = 231/1443$) vs. 9% ($n = 247/2747$), respectively. Compared with SLE patients without aPL, the overall pooled odds ratios (OR) for PH in aPL-positive SLE patients were 2.46 [95% confidence interval (CI), 1.84–3.28]. The risk of PAH associated with aPL in SLE patients was also significantly increased [OR = 3.06 (95% CI, 1.66–5.63)]. The risk of PH was the

highest for lupus anticoagulant (LA) [OR = 2.43 (95% CI, 1.53–3.87)] and IgG anticardiolipin antibodies (aCL) [OR = 3.12 (95% CI, 2.03–4.81)] while IgM aCL and anti β_2 -glycoprotein I antibodies were not significantly associated with PH [OR = 1.83 (95% CI, 0.91–3.67) and OR = 1.85 (95% CI, 0.55–6.27), respectively].

Conclusion: In SLE patients, aPL-positivity is associated with a dramatically increased risk for PH and PAH suggesting that an aPL-related mechanism other than venous thromboembolism can contribute to PAH. These findings warrant implementation of effective screening and treatment strategies.

Disclosure of Interest: None declared.

OR190

Obstetric antiphospholipid syndrome: early LMWH-induced variations of angiogenic factors predict pregnancy loss

Gris J-C^{1,2}, Bouvier S¹, Lavigne G¹, Nouvellon E¹, Mercier E¹, Galanaud J-P³, Quéré I³, Marès P⁴ and on behalf of NOHA network

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Background: Pregnancy loss can still occur in treated pregnant women with the obstetric antiphospholipid syndrome (oAPS). LMWH can mobilise angiogenic factors, among which placental growth factor (PGF) and the inhibitor soluble VEGF receptor (sFlt1). Antiphospholipid antibodies (aPLAbs) can impair the endometrial angiogenesis and trophoblastic functions through the modulation of angiogenic factors.

Aims: To study the LMWH-induced early variations of angiogenic factors and their prognostic value in newly pregnant oAPS women.

Methods: The study was performed in the 513 oAPS women included into the NOH-APS cohort, who began a new pregnancy treated with enoxaparin 40 mg + aspirin 100 mg daily (Blood 2014; 123: 404–13). Frozen plasma aliquotes from blood sampled on EDTA, obtained just before and 4 days after the beginning of LMWH injections (platelet count checks), were tested for PGF and sFlt1 concentrations (ELISA, R&D Systems, Abingdon, UK). Clinical outcomes were the occurrence of pregnancy loss subtypes (embryonic loss before 10 weeks, foetal loss, stillbirth). Multivariate logistic regression analysis models were computed.

Results: Enoxaparin induced significant PGF and sFlt1 increases. Variations in PGF were lower, and variations in sFlt1 higher, in women who developed foetal loss and in women who developed stillbirth. The ratio between variations of PGF and of sFlt1 were lower in case of foetal loss or stillbirth, with a dose-effect relationship between quartiles and clinical risk. This ratio remains an independent risk factor after adjustment for all covariates (previous pregnancy loss type, aPLAb triple positivity, ...).

Conclusion: In newly pregnant women with oAPS, the early LMWH-induced mobilisation of angiogenic factors can indicate the risk of subsequent foetal loss or stillbirth, not of embryonic loss.

Disclosure of Interest: None declared.

Venous thromboembolism diagnosis

OR191

Withholding anticoagulation after normal CTPA is safe in patients with a high clinical probability of PE in the absence of a prior episode of VTE

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Background: In patients with a low clinical probability (Wells clinical decision rule (CDR) score ≤ 4 points) of pulmonary embolism (PE) and normal D-dimer level, PE is safely excluded without computed tomography pulmonary angiography (CTPA). In the remaining patients with either a high CDR score (> 4 points) or an abnormal (≥ 500 ng mL⁻¹) D-dimer level, CTPA is indicated and in case of a normal CTPA PE is considered to be excluded and anticoagulation is withheld. However, in the 2014 ESC guideline (Konstantinides et al. Eur Heart J 2014;35:3045-3047) on acute PE, the safety of excluding PE in patients with a high CDR score and normal CTPA alone is considered to be unclear and whether these patients should be further investigated remains controversial.

Aims: To determine the safety of withholding anticoagulation in patients with a high CDR score in whom PE is excluded by normal CTPA alone and whether the safety is modified by the existence of a prior VTE.

Methods: Patient-level data from 4 prospective diagnostic management studies. Failure rates, defined as the 3-month incidence of venous thromboembolism (VTE) after PE was excluded, were pooled using a random-effects model for all patients, those with a high CDR score and patients with and without prior VTE and a high CDR score separately.

Results: Of 6148 patients were included with a pooled PE prevalence of 25%. The failure rate in all 4694 patients in whom PE was excluded was 1.2% (95%CI 0.5–2.6). In patients with high CDR score, the failure rate was 2.4% ($n = 1090$; 95%CI 1.1–4.9). For patients with a high CDR score without prior VTE, the failure rate was 1.8% ($n = 772$; 95%CI 1.1–3.1) and for those with prior VTE 3.6% ($n = 318$; 95%CI 2.0–6.4).

Conclusion: Withholding anticoagulation in patients with a high CDR score in whom PE is excluded by normal CTPA alone is safe for those without a prior episode of VTE. Whether patients with a high CDR score and a prior episode of VTE should be further investigated could be debated.

Disclosure of Interest: None declared.

OR192

Reproducibility of clinical events adjudications in a trial of venous thromboembolism preventionGirard P¹, Parent F^{2,3}, Gable B⁴, Sanchez O⁵, Durieux P⁶, Hausfater P⁷, Penalzo A⁸, Dambrine S⁴, Armand-Perroux A⁹, Beziaud M⁹, Nguyen J-P⁹, Meyer G⁵ and Roy P-M¹⁰

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Background: In many trials using clinical endpoints, an independent Clinical Events Committee (CEC) is in charge of adjudicating all clinical events relevant to the study endpoints according to predetermined criteria, to homogenize the interpretation of investigators data. However, very few data exist regarding the reproducibility of such adjudications.

Aims: To assess the reproducibility of CEC adjudications in a clinical trial.

Methods: The PREVENU study (NCT01212393) is a multicenter randomized trial of venous thromboembolism (VTE) prevention that included 15,182 medical patients. The main endpoint was the composite of symptomatic VTE, major bleeding, or unexplained sudden death at 3 months. A total of 2168 clinical events had to be adjudicated. The CEC comprised a chairman and 4 pairs of adjudicators. A random selection of 183 events (125 deaths, 40 bleeding events and 18 VTE events) that had been already adjudicated was blindly resubmitted to another pair of adjudicators. Adjudication agreement was measured by calculating Kappa values and their 95% confidence intervals (CI).

Results: Four events were excluded (incomplete adjudication forms). In the remaining 179 events, the overall Kappa value for the 3 components of the main endpoint was very good (0.84, CI: 0.77–0.91). When analyzed separately, Kappa values were very good for VTE (1, CI: NA), good for major bleeding (0.74, CI: 0.53–0.95), and moderate for deaths (0.60, CI: 0.40–0.81). The rates of disagreements in the causes of deaths were 0% (1 event) for fatal PE, 12% (1/8) for fatal bleeding, 56% (5/9) for unexplained sudden deaths and 7% (7/103) for other causes of death.

Conclusion: The reproducibility of adjudications for the composite endpoint, VTE and bleeding events was good or very good. The moderate reproducibility for unexplained sudden deaths, despite using strict predetermined criteria, reflects the limited availability of reliable information when fatal events occur after hospital discharge.

Disclosure of Interest: None declared.

OR193

DNA in plasma indicates disease extent and predicts mortality in patients with venous thromboembolismFuchs T^{1,2}, Jiménez-Alcázar M², Limacher A³, Méan M⁴, Renné T^{1,2}, Aujesky D⁴ and Lämmle B⁵

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Background: Venous thromboembolism (VTE) affects up to 5% of the population during their lifetimes and the incidence of VTE rises exponentially with age. Extracellular DNA may provide a target to improve the diagnosis and therapy of VTE. DNA enhances the activity of clotting factors and contributes to thrombus formation *in vitro* and *in vivo*.

Aims: We and others have previously identified elevated levels of DNA in plasma from patients or animals with deep vein thrombosis. The diagnostic and prognostic value of plasma DNA in VTE is not known. We hypothesized that levels of plasma DNA or DNaseI, the predominant DNA-degrading enzyme in plasma, correlate with the extent of VTE at diagnosis and are indicative of the clinical outcome.

Methods: To test our hypotheses we analyzed 863 patients of the SWIT-CO65 + cohort, a multicenter cohort study that prospectively enrolled consecutive patients aged ≥ 65 years with acute, symptomatic VTE. We quantified extracellular DNA using specific probes and DNaseI activity by zymography in patient plasma. Data was analyzed in competing risk and Cox-regression models, adjusting for relevant risk factors and periods of anticoagulation as a time-varying co-variant.

Results: Plasma DNA and DNaseI were positively ($P < 0.001$) and negatively ($P < 0.022$) correlated with the extent of VTE at enrollment, respectively. The median DNA/DNaseI ratio increased with VTE extent as indicated by distal DVT (231 ng mU⁻¹; $n = 71$), proximal DVT (410 ng mU⁻¹; $n = 195$), non-massive PE (667 ng mU⁻¹; $n = 589$) and massive PE (1546 ng mU⁻¹, $n = 8$). Neither DNA nor DNaseI were associated with the clinical outcomes VTE recurrence or major bleeding, but high DNA levels (top quartile) were predictive for mortality within 12 months post VTE diagnosis (adjusted HR 2.40, 95% CI 1.53–3.75, $P < 0.001$).

Conclusion: Increased DNA and decreased DNaseI in plasma are associated with the extent of VTE at time of diagnosis. Quantification of DNA in plasma may help identifying patients at risk of dying within months after acute VTE.

Disclosure of Interest: None declared.

OR194

Low end-tidal CO₂ significantly increases pretest probability of pulmonary embolismKline J¹, Verschuren F², Hogg K³, Roy P-M⁴, Penalzo A⁵ and Prentice D⁶

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Background: A meta-analysis found that alveolar deadspace from capnography as a test for pulmonary embolism had an area under the

summary receiver operating characteristic curve (ROC) of 0.84. However, alveolar deadspace calculation requires problematic arterial blood gas measurement. Limited data have been published on end-tidal CO₂ as a diagnostic test.

Aims: We hypothesized that etCO₂ would have a significant odds ratio (OR) for PE diagnosis in a multivariate model containing numerous known predictors of PE.

Methods: Secondary analysis of data from 6 individual studies of emergency department patients with suspected PE undergoing standardized diagnostic evaluation, including pulmonary vascular imaging and follow-up. Researchers prospectively collected etCO₂ and 16 other predictor variables (age, RR, HR, SBP, SaO₂, hemoptysis, smoking, lung disease, recent surgery, immobility, no prior cardiac disease, prior VTE, estrogen use, malignancy, suspected DVT, alternative diagnosis less likely). Parametric predictors (etCO₂, age, vital signs) were dichotomized after locating the optimal cutoff on ROC analysis. The diagnostic power of etCO₂ was tested with multivariate logistic regression to generate ORs and 95% CIs.

Results: From 2101 patients, 303 (14.4%) had PE. Of six parametric predictors, etCO₂ had the highest area under the ROC [0.72, (95% CI 0.69–0.75)] with optimal cutoff of 32.5 torr yielding a likelihood ratio (+) of 2.3 (2.0–2.5). Logistic regression revealed significant ORs for five predictors: etCO₂ < 32.5 torr 3.5 (2.4–5.1), suspected DVT 3.5 (2.1–5.9), alternative diagnosis less likely 3.4 (2.3–5.2), SaO₂ < 94.5% 2.2 (1.4–3.4), no prior cardiac disease 2.4 (1.1–5.9).

Conclusion: An end-tidal CO₂ < 32.5 torr significantly increases the probability of PE, independently of other predictor variables. As a non-invasive, inexpensive, widely available measurement, etCO₂ should be added to future predictive models for PE.

Disclosure of Interest: None declared.

OR195

A prospective algorithm incorporating limited and whole- leg assessment of the deep venous system in symptomatic outpatients (the Palladio study)

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Background: Compression ultrasonography (CUS) is the mainstay for the diagnosis of deep vein thrombosis (DVT) of the lower limbs. CUS can be extended to the entire venous system or limited to the proximal veins only. Extended CUS does not require repeat testing, limited CUS is simpler and easily available. The two approaches have been shown to be substantially equivalent.

Aims: We aimed to assess the accuracy of an algorithm combining extended and limited CUS.

Methods: In a multicenter, prospective cohort study, consecutive outpatients with clinically suspected DVT underwent D-dimer (DD) measurement and pre-test clinical probability (PTP) assessment. DVT was ruled out with no further testing in patients with PTP unlikely and negative DD (group 1). Patients with PTP likely or positive DD underwent limited CUS only (group 2), patients with PTP likely and positive DD underwent extended CUS (group 3). Patients in group 1 and patients with a negative CUS underwent a 3-month follow-up. Primary outcome was the incidence of objectively documented DVT or pulmonary embolism (PE) in patients in whom DVT was ruled out. A sample of 1100 patients was calculated on the assumption that the primary outcome would not exceed 1%, and the upper limit of the 95%

confidence intervals (CI) would not exceed 2%. Reported results are based on local adjudication only.

Results: Of 1162 eligible patients (median age 66 years, 60% females), 351 were in group 1, 401 in group 2, and 410 in group 3. Limited CUS was positive in 12 patients in group 2; extended CUS was positive in 200 patients in group 3. After the exclusion of 18 patients with protocol violations, the rate of the primary outcome in the whole cohort was 1.0% (95% CI, 0.5–1.9). The incidence of the primary outcome was 1.1% (95% CI, 0.3–2.9) in group 2 and 2.0% (95% CI, 0.6–5.2) in group 3.

Conclusion: An algorithm combining limited and extended CUS safely simplifies the approach to suspected DVT and reduces the diagnoses of low risk distal DVT.

Disclosure of Interest: None declared.

NOAC – Bleeding Risk Factors and Management

OR196

Influence of *ABCB1* genotype and of a P-gp drug inhibitor, clarithromycin, on dabigatran and rivaroxaban pharmacokinetic profiles in healthy volunteers

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Background: Drug interactions and inter-individual variations have been reported in healthy volunteers and patients receiving dabigatran etexilate and rivaroxaban. We hypothesized that genetic factors relating to their transport by P-glycoprotein (encoded by *ABCB1*), could affect their pharmacokinetic (PK) profiles.

Aims: The main objective was to evaluate the influence of the *ABCB1* genotype, on the PK profile of dabigatran and rivaroxaban in healthy volunteers. The secondary aim was to study the influence of a P-gp inhibitor, clarithromycin, on the PK of these drugs.

Methods: Sixty healthy Caucasian males selected on the basis of the *ABCB1* genotype (haplotype 2677-3435) i.e. 20 wild-type homozygous, 20 heterozygous and 20 mutated homozygous subjects were included in a randomised, open, crossover study comprising 3 single dose treatment periods: dabigatran 300 mg, rivaroxaban 40 mg, then combination of clarithromycin with dabigatran or rivaroxaban. Blood samples were taken between 0 and 24 h. Plasma concentrations of dabigatran and rivaroxaban were measured using a validated liquid chromatography-mass spectrometry method. PK parameters, including area under the concentration–time curve from time 0 to infinity (AUC) were derived from concentration–time data. A multivariate analysis using a

general linear mixed-effect model on log transformed AUC was performed to estimate the genotype and clarithromycin effects.

Results: Inter-individual variability CVs for AUC were of 77% for dabigatran and 63% for rivaroxaban. AUC increased by 1.25 and 1.28 ($P = 0.61$) with dabigatran and by 1.24 and 1.15 ($P = 0.15$) with rivaroxaban in *ABCB1* heterozygous and homozygous mutated subjects. Clarithromycin increased dabigatran AUC by 2 ($P = 0.00018$) and rivaroxaban AUC by 1.94 ($P < 0.0001$).

Conclusion: The high inter-individual variability and the marked effect of a P-gp inhibitor on both drug PK found in healthy volunteers suggest that the management of patients receiving rivaroxaban or dabigatran and multiple comedications may be challenging.

Disclosure of Interest: I. Gouin-Thibault Speaker Bureau of: Bayer, BMS-Pfizer, X. Delavenné: None declared, A. Blanchard: None declared, V. Siguret Speaker Bureau of: Bayer, BMS-Pfizer, P. Gaussem: None declared, C. Funck-Brentano: None declared, M. Azizi: None declared, P. Mismetti Speaker Bureau of: Boehringer Ingelheim, Bayer, BMS-Pfizer, Daiichi, M.-A. Lorient: None declared.

OR197

New oral anticoagulants in patients affected by high risk atrial fibrillation with history of intracranial hemorrhage

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Background: Intracranial hemorrhage (ICH) is the most devastating complication of anticoagulant therapy. Once ICH occurs, the decision of whether to resume anticoagulation or not is a therapeutic dilemma that requires balancing the risk of ICH recurrence against the risk of thromboembolic events.

Aims: To obtain a reliable estimate of the risks and benefits of New Oral Anticoagulants (NOACs) resumption in high risk Atrial Fibrillation (AF) patients with a history of ICH in order to design a wide national register.

Methods: Patients affected by high risk AF (CHADS Vasc ≥ 3) who presented to our centre in the last 16 months with a previous ICH were evaluated for resuming anticoagulant therapy. To all the patients with a favorable clinical and radiological evolution without other contraindications it was proposed to start NOACs. After one month a clinical reassessment was performed: in that occasion physicians could decide whether to perform a brain Computer Tomography to exclude hematoma growth or recurrent ICH, even in asymptomatic patients.

Results: Currently 13 patients have started Dabigatran, 15 Rivaroxaban and 10 Apixaban at the dose suggested by European Heart Rhythm Association guidelines. Of them 6 had a previous subarachnoid hemorrhage, 14 a subdural hematoma, 11 a deep hemispheric hemorrhage and 7 a lobar hemorrhage. At the time when ICH occurred 27 patients were being treated with Warfarin, 3 with Warfarin+Acetylsalicylic Acid (ASA), while 5 with ASA alone. The mean age at the baseline visit was 77.9 (SD ± 8.0) with a mean CHADS VASC of 5.0 (SD ± 1.3) and a mean Creatinine Clearance of 61.5 mL min⁻¹ (SD ± 17.7). Up to February 5th 2015 the mean follow up period has been 10 months: no intracranial event and major bleeding has been reported. 34 patients (89%) are on treatment. The rate of clinical relevant bleeding has been 19.0% per year.

Conclusion: Resumption of NOACs after ICH with a favorable evolution appears to be a reasonable alternative in frail high risk AF population.

Disclosure of Interest: None declared.

OR198

Intracranial bleeding with the new oral anticoagulant drugs

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Background: Dabigatran, rivaroxaban and apixaban were approved for stroke prevention and treatment of venous thrombosis in the past 4 years. Phase 3 studies reported a lower risk of intracranial bleeding compared to warfarin however there is little real-life data to show this.

Aims: We assessed time trends in oral anticoagulant-associated atraumatic intracranial bleeding from 2009 to 2013, and compared this to provincial oral anticoagulant (OAC) prescription trends.

Methods: ICD-10 codes were used to identify all atraumatic intracerebral bleeds presenting to our neurosurgical centre (covering a population of 1.3 million). Provincial prescription data for oral anticoagulants was obtained from IMS Brogan CompuScript Market Dynamics. The primary outcome was trend analysis of incident OAC-associated intracranial bleeding events per year. The secondary outcomes were comparisons between rate of OAC related bleeding and provincial prescription rates.

Results: Two thousand and fifty patients presented with atraumatic intracranial bleeds, median age 72, 51.5% male. Subdural and intracerebral hemorrhages accounted for 73% of all bleeds. 371 (18%) patients were prescribed OAC. There was an increasing trend over time in the rate of OAC-associated bleeding ($P = 0.009$) and non-OAC associated bleeding ($P = 0.063$). By the end of 2013, dabigatran, rivaroxaban and apixaban accounted for 16%, 17% and 3% of all provincial OAC prescriptions respectively. Warfarin consistently accounted for a disproportionately large number of OAC-associated bleeds compared to prescription prevalence. Dabigatran, rivaroxaban and apixaban each accounted for a non-significantly smaller proportion of OAC-associated bleeds when compared to prescription prevalence. There was no time trend in antiplatelet/anticoagulant co-prescription, and no association with renal impairment.

Conclusion: We found an increasing number of patients with OAC-associated intracranial bleeding over time, however there was a trend towards fewer intracranial bleeds associated with the new oral anticoagulants.

Disclosure of Interest: None declared.

OR199

Clinical impact and course of major bleeding with edoxaban vs. vitamin K antagonists

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Background: Edoxaban is a direct acting oral anticoagulant (DOAC)

that can be prescribed in a fixed dose regimen. The Hokusai VTE study revealed that, after initial treatment with heparin, edoxaban was

as effective as and safer than vitamin K antagonists (VKA) in the treatment of deep vein thrombosis and pulmonary embolism. There is little data about the clinical impact of bleeding events with edoxaban.

Aims: We blindly classified all major bleedings from the Hokusai VTE study for the severity of presentation and their clinical course.

Methods: All major bleeding events were classified blindly by three investigators, first separately and afterwards together. Pre-defined criteria were used. The first classification assessed the severity of clinical presentation. Category one comprised presentation without medical emergency, category three great medical emergency and category four fatal or almost fatal events. Category two was reserved for all other events. The second classification assessed clinical course. Category one consisted of only measures to treat discomfort, category two standard measures, category three elaborate measures to avoid death and category four was assigned when no lifesaving attempts were undertaken (death was unavoidable).

Results: Major bleeding occurred in 56 patients treated with edoxaban and 65 patients treated with VKA. The worst categories (three or four) of the clinical presentation were assigned to 46% of the major bleeding episodes in edoxaban recipients vs. 58% of the major bleeds in VKA recipients (odds ratio 0.62, 95% CI 0.30–1.27). Clinical course was classified as severe (category 3 or 4) in 23% of the edoxaban and 27% of the VKA associated bleeds (odds ratio 0.79, 95% CI 0.35–1.80).

Conclusion: Edoxaban associated major bleeding showed a trend towards a milder presentation and a milder course when compared to major bleeding events with VKA in patients treated for venous thromboembolism in the Hokusai VTE study.

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OR200

Characteristics and reversal strategies of real-world anticoagulation-related hemorrhage in the non-vitamin K antagonist oral anticoagulant era: preliminary data from a multicentre chart review

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Background: Non-vitamin K antagonist oral anticoagulants (NOACs) have expanded the options for antithrombotic therapy. While clinical

trial data demonstrate their favorable clinical outcomes following major hemorrhage, vitamin K antagonists (VKAs) were reversed in < 40% of cases, raising concerns on real-world applicability of this finding.

Aims: To describe baseline characteristics of patients presenting with NOAC- and VKA-related bleeding in routine care and the reversal and transfusion strategies associated with these bleeds.

Methods: We screened records of patients ≥ 66 years admitted to three tertiary care hospitals in Ontario, Canada who were discharged with hemorrhagic diagnoses, for association with use of an oral anticoagulant. Bleeds involving NOACs or VKA were compared with chi-square and *t* tests.

Results: A total of 12,988 charts from Oct 2010 to June 2014 were screened; 941 of those met criteria of anticoagulant-associated bleeding and were abstracted. Baseline characteristics between NOAC and VKA patients were similar across age (mean 81.1 vs. 80.8 for NOAC and VKA, respectively), sex (63% male vs. 59%) and CHA₂DS₂-VASc scores (mean 4.7 vs. 4.6). Proportion of bleeding which was gastrointestinal was higher for NOACs (69% vs. 42%, *P* < 0.01), but lower for intracranial causes (15% vs. 33%, *P* < 0.01). Red blood cell transfusions occurred in 61% and 39% of NOAC- and VKA-related bleeds, respectively. Among VKA bleeds, vitamin K (75%), PCC (41%) and FFP (22%) were the most commonly used reversal agents.

Conclusion: In this real-world cohort of hospital inpatients with oral anticoagulant-related hemorrhage, we observed similar baseline characteristics between NOAC- and VKA-bleed patients. While the distribution of anatomical site of bleeding was consistent with those reported in clinical trials, higher rates of VKA reversal attempts were observed when compared to trial data. Mortality rates and healthcare costs for this cohort will provide important future insights.

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Von Willebrand disease clinical

OR201

von Willebrand disease: does von Willebrand factor deficiency decrease the risk of cardiovascular disease?

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Background: von Willebrand factor (VWF) plays a critical role in platelet adhesion and aggregation after vascular injury and at sites of high shear rates. Elevated VWF levels are associated with an increased risk of ischemic cardiovascular events. Furthermore, animal studies indicate VWF deficiency is protective against atherosclerosis; however, it is unclear if this is the case in humans.

Aims: To compare the prevalence of cardiovascular disease (CVD) among patients with and without von Willebrand disease (VWD).

Methods: We performed a retrospective database analysis using discharge data among adults from the Nationwide Inpatient Sample (NIS) between the years 2009 and 2011. CVD was defined as ischemic heart disease (IHD), myocardial infarction (MI), ischemic cerebrovascular disease, or peripheral vascular disease. Prevalence calculations and statistical analyses used discharge-level weights provided by NIS to reflect national estimates. CVD was compared across groups using Rao-Scott chi-square test. Multivariable logistic regression was used to estimate the odds of CVD in patients with VWD after adjustment for age, gender, and CVD related risk factors (hypertension, hyperlipidemia, and diabetes mellitus).

Results: The prevalence of CVD in patients with VWD (unweighted $n = 7556$) was less than the prevalence of CVD in patients without VWD (unweighted $n = 19,918,970$), 15.0% vs. 26.0%, $P < 0.001$. IHD, 11.57% vs. 20.63, $P < 0.001$, and MI, 3.38% vs. 7.24% $P < 0.001$, were less common in patients with VWD than patients without VWD. VWD was associated with a decreased risk of CVD given age, gender, and CVD related risk factors, odds ratio (OR) = 0.84, 95% confidence interval (CI): 0.78, 0.90. The risk of IHD, OR = 0.85, 95% CI: 0.79, 0.92, and MI, OR = 0.68, 95% CI: 0.60, 0.77, was less following adjustment for age, gender, and CVD related risk factors.

Conclusion: These findings indicate the risk of CVD is decreased among patients with VWD and VWF deficiency may be protective against CVD. Prospective observational studies are warranted.

Disclosure of Interest: None declared.

OR202

Qualitative and quantitative modifications of von Willebrand factor in essential thrombocythemia: the role of platelets and non-ADAMTS13-dependent proteolytic processing

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Background: Essential thrombocythemia (ET) is characterized by increased platelet generation and thrombosis. Major bleeding are more rare and associated with extreme thrombocytosis. An acquired von Willebrand disease has been hypothesized based on a putative VWF consumption at extremely-high platelet count. However, whether and how VWF is modified in ET, even in patients with controlled platelet counts, remains poorly characterized.

Aims: We studied different VWF and platelet-associated parameters in ET patients treated according to current recommendations.

Methods: Sixty-nine ET patients, 69 matched controls and 10 reactive thrombocytosis (RT). VWF:antigen (Ag), activity (act), VWF multimers (VWFm), VWF:propeptide, plasma glycoalycin (GC), glycoprotein-V (GpV), ADAMTS-13, elastase and serum thromboxane (TX) B2 were measured.

Results: In ET, VWF:Ag was increased by $31 \pm 13\%$ vs. controls ($P < 0.01$) and was not influenced by blood group, while VWF:act was reduced by $21 \pm 12\%$ vs. controls and by $50 \pm 24\%$ vs. RT ($P < 0.01$). The ratio VWF(Act:Ag) was studied as an index of VWF integrity, and in ET were reduced by $35 \pm 17\%$ vs. controls and RT ($P < 0.001$) and significantly associated with immature or total platelet counts, GC, GpV, and TXB2. In multivariable analysis, only GC inversely predicted ET VWF(Act:Ag) ratios ($ps = -0.42$, $P = 0.01$). High-molecular-weight VWFm were variably reduced with atypical cleavage bands in ET only. The VWF proteolytic pattern of ET patients was incompatible with cleavage by ADAMTS-13 alone, at variance with RT. Platelet-associated ADAM-10 and ADAM-17 hydrolyzed VWFm *in vitro* showed patterns similar to ET samples.

Conclusion: In ET patients with controlled platelet counts, the VWF (Act:Ag) is decreased and predicted by GC, a product of platelet activation. *In vivo* platelet activation, which characterizes ET, might contribute to VWF alterations by ADAM-10 and ADAM-17 proteolytic activity.

Disclosure of Interest: None declared.

OR203

Impact of annexin A2 SNP on von Willebrand factor levels in patients with moderate and severe von Willebrand disease

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Background: von Willebrand Factor (VWF) is stored in Weibel Palade Bodies (WPB) present in endothelial cells. Exocytosis of WPB is regulated by the phospholipid binding protein Annexin A2 (AnxA2). Recently a common *AnxA2* SNP (rs17845226, c.346G>T) was identified, which causes a Valine to Leucine amino acid change and affects plasma vWF levels in controls. The frequency of the minor T allele in controls was 0.09.

Aims: In this study the prevalence of the minor T allele, associated with lower VWF levels in controls, and its effect on VWF plasma levels was analyzed in the nation-wide cross-sectional Willebrand in the Netherlands (WiN) population.

Methods: Plasma and DNA was collected from 745 type 1 and 2 VWD patients. rs17845226 was genotyped by Sanger sequencing. VWF Antigen (VWF:Ag), VWF activity (VWF:act) and vWF collagen binding activity levels (VWF:CB) were measured centrally. SNP data of a genome-wide association study (GWAS) control population ($n = 1502$) of the Nijmegen Biomedical Study (NBS) was used to calculate the Odds ratio (OR) with its 95% confidence interval for the WiN patients.

Results: The prevalence of the minor c.346 T-allele was shown to be increased in the WiN population compared to controls (OR = 1.49, 1.20–1.85), especially in patients with type 1 ($n = 409$; OR = 1.57, 1.20–2.02) and type 2B ($n = 39$; OR = 2.36, 1.16–4.61). In patients with type 1 VWD significant lower levels of VWF:Ag (TT: 35.8 IU dL⁻¹, GT: 34.7 IU dL⁻¹, GG: 42.2 IU dL⁻¹; $P = 0.014$) and VWF:act (TT: 43.6 IU dL⁻¹, GT: 43.0 IU dL⁻¹, GG: 52.4 IU dL⁻¹; $P = 0.043$) were determined in homozygous and heterozygous carriers of the SNP as compared to non-carriers. vWF:CB levels (TT: 42.4 IU dL⁻¹, GT: 40.5 IU dL⁻¹, GG: 48.9 IU dL⁻¹; $P = 0.054$) showed a trend to lower levels.

Conclusion: The *AnxA2* c.346 T-allele is associated with lower VWF among VWD patients in the WiN population and has an increased prevalence. This indicates that the severity and laboratory phenotype of VWD may be partly determined by the Annexin2 polymorphism.

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OR204

Variability in von Willebrand factor antigen (VWF:Ag) levels in prospective studies of the Zimmerman Program for the molecular and clinical biology of von Willebrand disease (ZPMCB-VWD) subjects

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Background: VWF levels vary over time, complicating the diagnosis of type 1 von Willebrand disease (VWD).

Aims: To determine the fidelity of the diagnosis of type 1 VWD, subjects in the ZPMCB-VWD are being re-evaluated over time.

Methods: We examined 173 subjects enrolled in ZPMCB-VWD in whom a family member was diagnosed with VWD at the referring center, including 113 Index Cases, 51 affected family members and 9 unaffected family members. At study entry, all subjects had factor VIII, VWF:Ag, VWF:RCO, VWF propeptide and VWF multimer analyzed at the clinical core lab. Phenotypic diagnosis was assigned using these entry labs as either quantitative VWF deficiency (VWF:Ag $\leq$ 50, $n = 89$), type 2 or 3 VWD ($n = 43$), type 1H (historic diagnosis, but entry labs did not confirm VWD, $n = 20$), or non-VWD ($n = 21$). Median age at enrollment was 19 (range 0–72) and at follow-up (FU) was 24 (range 3–77). Baseline levels were drawn from 2006 to 2013 and FU samples in 2012–2014; historic levels at referral centers were collected between 1982 and 2012.

Results: For the entire group, mean historic VWF:Ag was 38.2 U dL⁻¹, entry 45.6 IU dL⁻¹ and FU 42.0 IU dL⁻¹. Of the 45 patients with VWF:Ag $\leq$ 30, at FU, 6 (13%) had VWF:Ag in the low VWF range (VWF:Ag 31–50) and 1 (2%) had a level > 50 . Of the 40 subjects with VWF:Ag 31–50 at entry, 6 (15%) had FU levels > 50 and 1 (3%) had a level $\leq$ 30. Of subjects with VWF:Ag 51–70 ($n = 20$), 8 (40%) had FU levels of 31–50 and no FU level was $\leq$ 30. Finally, of subjects with entry VWF:Ag > 70 ($n = 27$), only 1 had a FU level in the 31–50 range.

Conclusion: In this selected group of patients, the fidelity of a diagnosis of quantitative VWF deficiency is a function of the VWF:Ag at the

time of study entry, with 95% of patients continuing to show type 1 VWD if the entry level was ≤ 30 , but some patients with levels between 51% and 70% showing deficiency upon repeat study. For patients in whom quantitative VWF deficiency is being considered, the initial VWF:Ag level may serve as a guide to whether repeated studies will assist in diagnosis.

Disclosure of Interest: None declared.

OR205

Characterization of a new genetically-engineered von Willebrand disease type 2B mouse model

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Background: von Willebrand disease-type 2B (VWD2B) is characterized by gain-of-function mutations localized in Von Willebrand factor (VWF) A1 domain. We engineered the first knock-in murine model for type 2B VWD in the murine *Vwf* gene leading to the p.V1316M/VWF mutation.

Aims: To characterize the hemostatic phenotype of this VWD2B mouse model.

Methods: Hematological parameters were determined for heterozygous (HET) and homozygous (KI) VWD2B mice. Platelet function was assessed *in vivo* in a tail bleeding assay and in a thrombosis model, and *in vitro* in thrombus formation over collagen under flow conditions and in platelet aggregation. Platelet adhesion to secreted VWF by endothelial cells was also evaluated *in vivo*.

Results: Platelet count was decreased for HET (10%) and KI (50%) mice, and KI platelets exhibited a 44% increase in size. In tail bleeding assays, bleeding time was variable for HET mice (45% over 10 min), while infinite for KI mice. In a FeCl₃-induced *in vivo* thrombosis model, vessel occlusion was variably delayed in HET mice, while no occlusion ever occurred in KI mice. *In vitro*, HET and KI platelets displayed a defective thrombus formation in a whole-blood perfusion assay. Aggregation was decreased at low doses of agonists in HET platelets and abolished in KI platelets. At high doses, aggregation of HET platelets was normal, but still defective in KI platelets. Finally, surprisingly after *in vivo* stimulation of endothelial cells, platelet-decorated VWF strings were enhanced in KI mice. Moreover, the lifetime of VWF strings was prolonged, suggesting resistance against ADAMTS13-mediated proteolysis.

Conclusion: Our genetically-engineered VWD2B mice represent a new and useful model to study the consequences of spontaneous VWF-platelet interactions and the physiopathology of the human disease.

Disclosure of Interest: None declared.

Platelets – I

OR206

Single cell tracking reveals autonomous locomotion as a platelet function facilitating thrombus reorganization

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Background: Platelets are anucleated cells that circulate in mammalian blood relentlessly scanning the vasculature for damage of the endothelial surface. Once recruited from the blood stream, platelets are generally considered to be immotile, attaching firmly and spreading on whatever subendothelial matrix or plasma protein they first encounter. However due to the high platelet density within a thrombus and the small platelet size, we actually know only very little about the exact behavior of individual platelets within a forming thrombus.

Aims: Here we aimed to identify the dynamic behavior of individual platelets during thrombus formation.

Methods: Using 2-photon intravital microscopy we identified and tracked single multicolored platelets during thrombus formation following vascular injury *in vivo*. Live cell imaging of isolated platelets was used to quantify the morphodynamics of platelet locomotion *in vitro*.

Results: By single cell tracking we demonstrate autonomous locomotion of activated platelets within their physiological environment. Platelets polarize, become motile and migrate while undergoing the typical cycles of cell migration established for other cell types. We show that the major intra- and extracellular factors governing platelet migration include actin-mediated protrusion and integrin-dependent adhesion formation at the leading edge, balanced by calcium-mediated myosin IIa-dependent contraction and adhesion release at the trailing edge to maintain polarization and forward movement. Governed by the local adhesiveness of the microenvironment, platelet migration facilitates the redistribution of individual platelets towards regions of higher adhesiveness, thereby reorganizing thrombi *in vivo*.

Conclusion: Here we identify anucleated platelets as motile cells and demonstrate autonomous platelet migration as a platelet function regulating thrombus formation.

Disclosure of Interest: None declared.

OR207

More than just red cells: how do hemostatic thrombi prevent the loss of plasma-borne molecules?

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Background: Hemostatic thrombi formed after penetrating injuries have a structure in which a core of fully-activated, densely-packed platelets is overlaid with a shell of less-activated, loosely-packed platelets. Past studies show that this structure is able to halt red cell loss at a time when only small numbers of platelets and little fibrin have accumulated.

Aims: Based on the hypothesis that escape of plasma-borne and platelet-derived molecules affects wound healing and inflammatory responses to injury, we examined the relationship between thrombus structure and the formation of a seal sufficient to limit the delivery of bioactive molecules into surrounding tissues.

Methods: Mice were infused with albumin linked to caged fluorescein (cAlb) prior to laser injury in cremaster muscle venules. Intravital microscopy tracked thrombus formation and plasma loss.

Results: cAlb extravasation continued well after red cell loss ended, decreasing with time, but still detectable after 20 min. During this period fibrin accumulated and thrombus core-and-shell architecture emerged. Inhibition of platelet accumulation via infusion of eptifibatide resulted in enhanced plasma loss. Hirudin had little effect despite abolishing fibrin deposition. Blocking ADP receptors stripped away the thrombus shell, increasing cAlb loss by 40% 3 min after injury, while introduction of G_{i2} gain of function mutation increased the size of the shell without significantly affecting plasma loss. Finally, thrombi formed in mice with a defect in clot retraction showed reduced packing density in the core and a 30% increase in plasma leak.

Conclusion: These results show that the hemostatic seal formed after penetrating vascular injuries is initially much less effective in limiting the loss of plasma than red cells, and suggests that in this model the entire core-and-shell architecture is needed for maximal control of plasma loss. Fibrin accumulation, in contrast, appears to play only a minor role in this aspect of hemostasis.

Disclosure of Interest: None declared.

OR208

Dicer, the key enzyme of RNA interference, is regulated by thrombin stimulation in human platelets

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Background: Although anucleate, circulating platelets contain messenger RNAs (mRNA) and microRNAs (miRNA) and synthesize new proteins upon activation. Platelets contain functional Dicer and Argonaute 2, able to process miRNA precursors (pre-miRNA) and to control the expression of specific transcripts. miRNAs of human platelets can be up or down-regulated upon thrombin stimulation.

Aims: Our aim was to evaluate whether *de novo* synthesis of Dicer occurs in platelets upon thrombin stimulation and to show if this may modify miRNAs and mRNAs profile.

Methods: Platelets from healthy human volunteers were purified by immunomagnetic beads, resuspended at $0.5 \times 10^6 \mu\text{L}^{-1}$ and activated with thrombin for different intervals. Dicer protein expression was analysed by western blotting in lysed platelets and by flow cytometry in permeabilized platelets. The translational inhibitor puromycin and radiolabelling of proteins by S³⁵-methionine were used to confirm *de novo* protein synthesis. Radioactive Dicer isolated by immunoprecipitation was quantified by liquid scintillation counting. Expression of mRNA for P2Y₁₂ was quantified by RT-PCR to evaluate the activity of Dicer because it is a target of the most abundant miRNA present in platelets, mir-223.

Results: Dicer protein and mRNA are present in resting human platelets. Activation with thrombin induces a rapid increase in the expression of Dicer protein in platelets, already evident at 5 min and steadily increasing until 60 min. The increase of Dicer was blocked by pretreatment with puromycin. S³⁵-methionine was incorporated into Dicer in activated platelets, confirming *de novo* synthesis. Thrombin induced a down-regulation of P2Y₁₂ which correlated with Dicer increase.

Conclusion: Thrombin induces a rapid and marked *de novo* synthesis of Dicer in human platelets. Newly synthesized Dicer is functional because it modifies the expression of a target platelet mRNA (P2Y₁₂ mRNA). Our results add an additional level of complexity to the control of gene expression in human platelets.

Disclosure of Interest: None declared.

OR209

Replication of platelet reactivity genome-wide association single nucleotide polymorphisms in the Caerphilly Prospective Study

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Background: Recently, our group completed the largest genome-wide association study (GWAS) on platelet reactivity. We showed associations of many single nucleotide polymorphisms (SNPs) in or near *PEAR1*, *MRV1*, *RGS18*, *ARHGEF3*, *JMJD1C*, among others.

Aims: Our goal was to replicate the association of 13 SNPs in our prior GWAS in the Caerphilly Prospective Study (CaPs), a UK population-based cohort of middle-aged men.

Methods: Platelet reactivity was measured by shear-stress, ADP in whole blood, ADP in platelet-rich plasma (PRP), and thrombin in PRP. Platelet count (PLT) and mean platelet volume (MPV) were also collected. 13 SNPs, with genome-wide or suggestive associations in our prior GWAS, were genotyped using KASP and called with SNP-Viewer. Approximately 1350 subjects had both platelet-related and SNP data. Platelet reactivity and count measures were analyzed for additive and recessive genetic associations, correcting for exam and age.

Results: The strongest platelet reactivity associations were seen between *PEAR1* SNP rs12566888 and reactivity to thrombin ($P = 1.8 \times 10^{-6}$) and ADP ($P = 9.2 \times 10^{-6}$). There were also associations of *RGS18* SNP rs4128880 with ADP reactivity ($P = 1.9 \times 10^{-4}$). These SNPs, as well as other platelet reactivity specific SNPs, did not show association with PLT or MPV. However, we did replicate associations of rs10761741 in *JMJD1C* ($P = 2.0 \times 10^{-8}$) and rs12485738 in *ARHGEF3* ($P = 1.1 \times 10^{-4}$) with MPV.

Conclusion: We replicated associations of SNPs in *PEAR1* and *RGS18* with platelet reactivity as well as SNPs in *JMJD1C* and *ARHGEF3* with MPV. We report the first genetic associations with thrombin reactivity at the *PEAR1* locus, now genetically linked to ADP, epinephrine, thrombin, and anti-platelet therapeutic response. Strikingly, platelet reactivity SNPs did not show associations with PLT or MPV, suggesting these traits are influenced by distinct genetic factors. We will examine these genetic factors further by completing a GWAS of platelet function and count in CaPs.

Disclosure of Interest: None declared.

OR210

Vps33b regulates VWF positive vesicles transportation to proplatelet

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Background: Vacuolar protein sorting protein 33b (Vps33b) mutations cause ARC syndrome with a common feature of lack of α -granules in the patients' platelets. Although a putative mechanism of Vps33b mostly comes from the study of yeast homologue Vps33, the precise

function of Vps33b involvement in α -granule formation remains unknown.

Aims: To study the mechanisms of Vps33b involved in α -granule formation.

Methods: Conditional Vps33b knockout mice were developed to investigate the function(s) of Vps33b in platelet α -granule formation. Co-IP, MS and immunoblotting were used to identification the Vps33b complex. IF images were obtained to illustrate the localization of Vps33b complex with α -granule related vesicles.

Results: We found that early embryonic deletion of Vps33b was lethal. PF4-Cre driven megakaryocytic Vps33b deletion significantly eliminated Vps33b expression in platelets, but had no effect on platelet α -granule formation. Tamoxifen induced HSC-specific Vps33b deletion completely depleted Vps33b in platelets and caused the absence of α -granules and increased numbers of vacuoles in platelets. Vps33b association with Vps16b, α -tubulin and Sec22b was identified by co-IP, MS and immunoblotting. Also, pull-down experiments revealed that Vps16b bound to intact Vps33b; in contrast, α -tubulin and Sec22b interacted with the sec1-like domains of Vps33b. Furthermore, vWF positive vesicles obviously spread along the Vps33b complex in differentiated MEG-01 cells. Transportation of the vWF positive vesicles to proplatelets was arrested in the differentiated primary Vps33b deficient megakaryocyte, suggesting that Vps33b complex regulates α -granule formation by the incorporation of the vWF-positive vesicles into the proplatelets.

Conclusion: The results demonstrate the previously undocumented new mechanisms of Vps33b in platelet α -granule formation. Vps33b apparently regulates vWF-positive vesicles transportation to proplatelet in differentiated megakaryocyte, thus plays an essential role in platelet α -granule biogenesis.

Disclosure of Interest: None declared.

Hemophilia – novel Treatments

OR211

Personalization of treatment regimens for active patients: a comparison of factor VIII and extended half-life treatment regimens

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Background: Personalizing FVIII treatment includes matching infusions with patient lifestyle. With new extended half-life (EHL) FVIII products, it is important to identify which patients may be most appropriately treated on different treatment products/regimens.

Aims: Estimate relative bleeding risk between prophylaxis regimens using recombinant FVIII (rFVIII) and BAX 855, an investigational EHL-rFVIII, among different patient physical activity profiles.

Methods: A literature-based model was developed with FVIII levels estimated from a one-compartment pharmacokinetic model (Collins 2010). Half-life and incremental recovery for rFVIII and EHLF-rFVIII were taken from the BAX 855 pivotal trial. Three prophylaxis regimens were used: rFVIII 30 IU kg⁻¹ every other day (EOD), rFVIII 35 IU kg⁻¹ 3× per week, and BAX 855 45 IU kg⁻¹ 2× per week. Activity categories were from the NHF 'Playing It Safe' brochure. Three hypothetical activity profiles were evaluated: Consistently Active (M-Sun: Type 2), Regular Exerciser (M-F: Type 2, Sat-Sun: Type 1), and Weekend Warrior (M-F: Type 1, Sat-Sun: Type 3). Bleeding risk by activity category and FVIII was calculated using odds ratios from Broderick 2012. For each regimen, the infusion schedule with the lowest bleeding risk for the activity profile was used. The relative bleeding risks were compared between regimens.

Results: For the Regular Exerciser, rVIII 3× per week was estimated to have a 2% and 8% lower relative bleeding risk than BAX 855 and rFVIII EOD respectively. For the Weekend Warrior, BAX855 was estimated to have a 12% and 13% lower relative bleeding risk for rFVIII 3× per week and rFVIII EOD respectively. For the Consistently Active patient rFVIII EOD was estimated to have a 5% and 8% lower relative bleeding risk for both rFVIII 3× per week and BAX 855 respectively.

Conclusion: The model suggests different patient types may be better suited for rFVIII while others for BAX 855 2× per week. Physicians should consider personalizing treatment using different products and regimens for different patient types.

Disclosure of Interest: None declared.

OR212

Clinical evaluation of novel recombinant glycopegylated FVIII (turoctocog alfa pegol, N8-GP): efficacy and safety in previously treated patients with severe hemophilia A – results of pathfinder™2 international trial

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Background: N8-GP (turoctocog alfa pegol) is a novel glycoPEGylated long-acting rFVIII product developed for safe and effective prophylaxis and treatment of bleeding episodes in patients with hemophilia A, with less frequent dosing in comparison to regular FVIII products.

Aims: This phase 3 trial (NCT01480180 [www.clinicaltrials.gov]) was a multi-national, open-label trial evaluating safety, pharmacokinetics and clinical efficacy of N8-GP when administered for prophylaxis and treatment of bleeds in previously treated patients (PTP) (≥ 12 years) with severe hemophilia A (FVIII < 1%).

Methods: Patients were allocated (non-randomized) to receive N8-GP for prophylaxis (PPX) at 50 U kg⁻¹ every 4th day, or for on-demand treatment (OD). Bleeds were to be treated with doses between 20 and 75 U kg⁻¹ in both treatment arms.

Results: In the trial, 175 patients received N8-GP for PPX and 12 patients received OD when bleeds occurred (one patient changed treatment regimen, and is included in both the prophylaxis and on-demand arm). Patients were treated for up to 21 months, resulting in median annualized bleeding rates of 1.3 for patients treated prophylactically and 30.9 for patients on OD. A total of 40% of the patients treated prophylactically did not experience any bleeds. N8-GP was used to treat a total of 968 bleeds in 117 patients across treatment arms, of which 95.5% were resolved with ≤ 2 injections of N8-GP. The pharmacokinetic analysis documented a single dose half-life of 18.3 h for N8-GP. Treatment with N8-GP was found to be safe and well tolerated. One out of 186 patients developed FVIII inhibitors after 93 N8-GP exposure days, revealing a frequency in line with expectations in a population of PTP.

Conclusion: N8-GP was effective in the treatment of bleeds and in prophylaxis of bleeding and appeared safe and well tolerated.

Disclosure of Interest: P. Giangrande Grant/Research Support from: Novo Nordisk, Consultant for: Novo Nordisk, P. Chowdary Grant/Research Support from: Novo Nordisk, Pfizer, CSL Behring, Consultant for: Novo Nordisk, Pfizer, CSL Behring, Baxter, Biogen Idec, Bayer, S. Enhrenforth Employee of: Novo Nordisk, H. Hanabusa Paid Instructor at: Baxter Healthcare, Novo Nordisk, Bayer, Pfizer, Biogen Idec and KaketsuKen, Speaker Bureau of: Baxter Healthcare, Novo Nordisk, KaketsuKen and Biogen Idec, F. Leebeek: None declared, S. Lentz Grant/Research Support from: Novo Nordisk, Consultant for: Novo Nordisk, L. Nemes: None declared, L. Poulsen Consultant for: Bayer Health Care, Speaker Bureau of: Bayer Health Care, Pfizer, E. Santagostino Grant/Research Support from: Pfizer, Bayer, Baxter, Novo Nordisk, CSL Behring, Grifols, Biotest, Consultant for: Pfizer, Speaker Bureau of: Biotest, Octapharma, C. You: None declared, W. H. Clausen Employee of: Novo Nordisk A/S, J. Oldenburg Grant/Research Support from: Baxter, Bayer, Biogen Idec, Biotest, CSL-Behring, Grifols, Novo Nordisk, Octapharma, Swedish Orphan Biovitrum and Pfizer, Paid Instructor at: Baxter, Bayer, Biogen Idec, Biotest, CSL-Behring, Grifols, Novo Nordisk, Octapharma, Swedish Orphan Biovitrum and Pfizer, Speaker Bureau of: Baxter, Bayer, Biogen Idec, Biotest, CSL-Behring, Grifols, Novo Nordisk, Octapharma, Swedish Orphan Biovitrum and Pfizer.

OR213

A subcutaneously administered investigational RNAi therapeutic (ALN-AT3) targeting antithrombin for treatment of hemophilia: interim phase 1 study results in patients with hemophilia A or B

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Background: Hemophilia A and B are characterized by defect thrombin generation (TG) and in the presence of normal levels of endogenous anticoagulants, a deficiency of factor VIII or IX results in hemostatic imbalance and a bleeding phenotype.

Aims: ALN-AT3 is a subcutaneous (S.C.) investigational RNAi therapeutic targeting antithrombin (AT) with the purpose of increasing TG and thereby restoring hemostatic balance in hemophilia.

Methods: A Phase 1 study (NCT02035605) in healthy volunteers and subjects with severe or moderate hemophilia A or B. Part A is a single ascending dose study in healthy volunteers. Parts B and C are multiple ascending dose studies in hemophilia patients with weekly and monthly dosing. Primary endpoints are safety and tolerability. Secondary endpoints are PK, AT knockdown (KD) and change in TG and whole blood (WB) clot formation. Bleed pattern and control are exploratory endpoints.

Results: Part A enrolled 4 subjects, randomized to 30 µg kg⁻¹ ALN-AT3 or placebo (3:1). No SAEs or injection site reactions were observed. A total of 5 mild AEs were recorded. The maximum AT KD was 28% with mean maximum AT KD of 19 ± 4.4% (mean ± SEM, P < 0.01). This led to up to 152% increase in peak TG with mean

maximum increase in peak TG of $138 \pm 8.9\%$ ($P < 0.01$). The AT KD was stable and durable for over 60 days. In Part B, several cohorts of 3 hemophilia patients will receive 3 weekly doses of ALN-AT3. Three weekly doses of 15 and $45 \mu\text{g kg}^{-1}$ ALN-AT3 have been generally safe and well tolerated, and resulted in up to ~80% AT KD with increased TG and improved WB clot formation. Updated results from Parts B and C will be presented.

Conclusion: Emerging clinical data suggest that targeting AT could be a promising approach for restoring hemostatic balance in hemophilia. The potential for low volume S.C. administration, infrequent dosing, and applicability to persons with hemophilia who have inhibitors, make ALN-AT3 a particularly encouraging investigational therapy.

Disclosure of Interest: None declared.

OR214

BAY 81-8973 prophylaxis efficacy in patients with severe hemophilia A: analyses of annualized bleeding rate outcomes in the LEOPOLD I trial

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Background: BAY 81-8973 is Bayer's new full-length recombinant factor VIII (rFVIII) manufactured with technologies to improve consistency in glycosylation and expression for optimal clinical performance in treating hemophilia A.

Aims: To examine dose, dosing regimens, annualized bleeding rate (ABR), and time to bleed after prophylaxis infusion in subgroups of patients receiving BAY 81-8973 prophylaxis in the open-label, randomized LEOPOLD I trial.

Methods: Patients meeting LEOPOLD I eligibility criteria (aged 12–65 years, severe hemophilia A, ≥ 150 exposure days to FVIII, no inhibitors) received BAY 81-8973 prophylaxis ($20\text{--}50 \text{ IU kg}^{-1} 2\text{--}3\times$ per week per investigator decision) for 12 months.

Results: Patients treated $2\times$ per week ($n = 18$) vs. $3\times$ per week ($n = 44$) during the study (mean age, 37 vs. 29 years) experienced a median of 2.0 vs. 4.0 joint bleeds in the last 12 months; 89% vs. 77% were previously treated with prophylaxis, and 56% vs. 77% had a target joint at baseline. Patients with 0 ($n = 16$) vs. ≥ 1 bleed ($n = 46$) during the study (mean age, 37 vs. 30 years) had a median of 2.0 vs. 7.0 bleeds in the last 12 months, and 63% vs. 74% had a target joint at baseline; median annual dose was 4550 vs. 4886 $\text{IU kg}^{-1} \text{ year}^{-1}$ during the study, and 38% vs. 26% were treated $2\times$ per week. Median ABR was 1.0 for $2\times$ per week prophylaxis vs. 2.0 for $3\times$ per week; 2.0 for study months 0–6 vs. 0.0 for months 7–12 (all patients); 2.3 for low-dose ($20\text{--}30 \text{ IU kg}^{-1} \text{ infusion}^{-1}$; $n = 38$) vs. 1.0 for high-dose prophylaxis ($35\text{--}50 \text{ IU kg}^{-1} \text{ infusion}^{-1}$; $n = 24$); and 2.0 for $< 80 \text{ IU kg}^{-1}$ ($n = 14$) vs. 1.0 for $\geq 80 \text{ IU kg}^{-1}$ ($n = 48$) weekly prophylaxis dose. Median dose per infusion ($\text{IU kg}^{-1} \text{ infusion}^{-1}$) was 31.5 vs. 33.9 for patients treated $2\times$ per week vs. $3\times$ per week. Mean time to bleed after infusion was 2.3 days for $2\times$ per week vs. 1.8 days for $3\times$ per week treatment.

Conclusion: BAY 81-8973 prophylaxis $2\times\text{--}3\times$ per week improved outcomes across several subgroups of patients with severe hemophilia A. Prestudy bleed rate was the best predictor for an on-study ABR of 0.

Disclosure of Interest: M. E. Mancuso Consultant for: acted as a consultant and member of advisory boards for Bayer, Baxter, Pfizer, and CSL Behring, Speaker Bureau of: Bayer and Novo Nordisk, H. Beckmann Employee of: Bayer Pharma AG, M. Maas Enriquez Employee of: Bayer Pharma AG.

OR215

Factors influencing the pharmacokinetics of pegylated recombinant factor VIII (BAX 855) administered for prophylaxis in previously treated patients with severe hemophilia A

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Background: BAX 855 is a novel polyethylene glycol (PEG)ylated full-length recombinant factor VIII, built on the PEG-rAHF-PFM (ADVATE) protein, that has been developed to offer a safe and efficacious option in treating hemophilia A while extending the half-life ($T_{1/2}$). The pharmacokinetics (PK) of FVIII exhibit considerable inter-patient variability, thus determination of the patient factors influencing the PK of BAX 855 may assist in individualized dosing.

Aims: To assess the PK of BAX 855 at initial administration and after ≥ 50 exposure days (EDs).

Methods: Adolescent (12 to < 18 years) and adult (18–65 years) previously-treated male patients with severe hemophilia A received BAX 855 as prophylaxis ($45 \pm 5 \text{ IU kg}^{-1}$ twice weekly), or on-demand ($10\text{--}60 \text{ IU kg}^{-1}$). PK was assessed at the initial dose and after ≥ 50 EDs in a subgroup on prophylaxis ($n = 25$ planned, including ≥ 6 adolescents). Subjects' informed consent and appropriate ethics committee approvals were obtained.

Results: The PK analysis included 26 subjects; median doses were 45.04 IU kg^{-1} (initial infusion) and 45.83 IU kg^{-1} (repeat PK infusion). $T_{1/2}$, mean residence time (MRT), and area under the concentration curve from 0 to infinity ($\text{AUC}_{0\text{--}\infty}$) of BAX 855 were positively correlated with VWF:Ag concentration (Spearman coefficients after initial treatment: 0.72, 0.73 and 0.78). $T_{1/2}$ was slightly shorter in adolescents than in adults (mean [SD] after the first dose: 13.43 h [4.051] vs. 14.69 h [3.793]), as was MRT (17.96 h [5.490] vs. 20.27 h [5.234]). Overall, the circulating time of BAX 855 was slightly longer after ≥ 50 EDs than after the initial dose, as shown by the mean [SD] ratios for $T_{1/2}$ (1.181 [0.4730]) and MRT (1.101 [0.2567]). All results shown were determined using the one-stage clotting assay; similar trends were observed with the chromogenic assay.

Conclusion: The PK of BAX 855 is influenced by VWF:Ag levels, age group and possibly by length of exposure.

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Acquired disorders – Clinical – II

OR216

Comparative analysis of von Willebrand factor profiles in left ventricular assist device and total artificial heart recipients

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Background: Bleeding remains a significant clinical challenge during mechanical circulatory support & underlying mechanisms are incompletely understood. Functional impairment in von Willebrand Factor

(vWF) due to loss of high molecular weight multimers (MWM) produces acquired von Willebrand syndrome (vWS) & has been reported with left ventricular assist devices (LVADs). Little is known about vWF multimers with total artificial hearts (TAHs).

Aims: We compare vWF profiles in patients with LVADs & TAHs using a comprehensive vWS panel.

Methods: Hospitalized LVAD & TAH patients with vWS workup at our center from 2013 to 2014 were retrospectively analyzed. vWS workup included vWF activity (Ristocetin Cofactor, R:CO), vWF antigen (VWF Ag), ratio of R:CO to vWF Ag, & quantitative vWF multimer analysis. The relationships between type of multimer loss & type of device were tested for significance using Fisher's exact test.

Results: Twenty-one patients underwent vWS workup: 11 with continuous flow LVADs & 10 with TAHs. All had either normal (48%) or elevated (52%) R:CO, normal (38%) or elevated (62%) vWF Ag, & the majority (85%) were disproportional (ratio < 1: relative qualitative defect). Quantitative vWF multimer analysis showed abnormal patterns in 10/11 (91%) of patients with LVADs: 6 with high MWM & 4 with highest MWM loss. With TAH, 9/10 patients had abnormal patterns: all with highest MWM loss. There were significant relationships between high MWM loss in LVAD patients ($P = 0.01$) & highest MWM loss in TAH patients ($P = 0.02$). Increased low MWM were detected in 20/21 (90%) patients (mean $29 \pm 3\%$; normal range, 16–24%).

Conclusion: Using quantitative vWF multimer analysis, we detected abnormalities after LVAD or TAH that would be missed with measurements of vWF level alone: loss of high MWM predominantly in LVAD, loss of highest MWM in TAH, & elevated levels of low MWM in both. This is the first study to describe TAH-associated highest MWM loss, which may contribute to bleeding.

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OR217

Relapse pattern and long term outcomes in 111 subjects with acquired hemophilia A

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Background: Acquired haemophilia A (AHA) is a rare autoimmune bleeding disorder caused by antibodies against factor VIII. Despite significant morbidity and mortality, most patients achieve remission with immunosuppressive therapy. Little is known about patterns of relapse and outcomes.

Aims: Long-term follow-up data from the Quebec Reference Centre for Inhibitors (QRCI) were analyzed to describe factors predictive of relapse and their influence on survival in AHA.

Methods: The QRCI was created by the Health Ministry to oversee the use of bypassing agents and to report on health outcomes in patients receiving these agents. Diagnostic criteria for active AHA were: FVIII: C < 0.30 UI mL⁻¹ and FVIII inhibitor ≥ 0.6 Bethesda Unit (BU). Complete remission (CR) was defined as FVIII:C > 0.50 UI mL⁻¹ and/or FVIII inhibitor < 0.6 BU while not on immunosuppression. Paired-sample *t*-test was used to compare patients' characteristics.

Results: Between 2000 and 2012, 100/111 registered patients (90%), median age 73 years, achieved CR within a median of 45 days and were followed up for a median time of 25.6 months. Of those, 14% presented one or more relapses (median time to first relapse 13.4 months [4.1–53.3 months]). Patients with relapse were older ($P = 0.054$), mostly male ($P = 0.083$) and more frequently suffering from auto-immune or lymphoproliferative disease ($P = 0.008$). Baseline factor VIII and inhibitor titres did not differ between the 2 groups ($P = 0.637$). The initial immunosuppressive regimen was not predictive of the risk of relapse. Among patients achieving remission at presentation, the overall survival (OS) was the same ($P = 0.541$), with or without relapse. All but one relapsing patients achieved a subsequent remission and only one patient had a fatal bleed during an episode of relapse.

Conclusion: Relapse of AHA is infrequent once CR is achieved and not associated with a worse OS. Subsequent CR can be obtained in most cases. Our study shows the importance of a long-term follow-up.

Disclosure of Interest: None declared.

OR218

Thrombin generation varies with fibrosis score in patients with well-compensated liver cirrhosis of mixed aetiology

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Background: Fibrosis is a hallmark of chronic liver disease. Liver fibrosis can progress to cirrhosis; nodule formation, reduced liver cell regeneration, and loss of liver cell function. Until recently, there was no non-invasive method of staging cirrhosis. Now, transient elastography (FibroScan™), a liver stiffness measurement (LSM) can be generated in compensated cirrhotic patients and associates with portal hypertension and clinical outcomes. While decompensated cirrhosis is associated with coagulation abnormalities that increase the risk of thrombosis and bleeding, it is unclear if or when coagulation abnormalities appear in compensated cirrhosis.

Aims: Measure and compare thrombin generation with disease severity in patients with cirrhotic liver disease of mixed aetiology.

Methods: Compensated cirrhotic patients were recruited at the Mater Hospital, Dublin. FibroScan™ imaging was used to calculate LSM. Thrombin generation in platelet poor plasma (1 pM TF) was measured using a Fluoroskan Ascent plate reader with Thrombinoscope software.

Results: Thrombin generation was compared between patients with a LSM below and above 35 kPa (16 ± 1 , $n = 47$ vs. 53 ± 5 , $n = 10$). Higher LSMs reflect more fibrosis and portal hypertension. A cutoff of 35 kPa was chosen as it predicted short term liver decompensation. Despite comparable clotting times, thrombin generation was impaired in patients with a LSM > 35 kPa. There was a decrease in peak thrombin generation (194 ± 9 vs. 133 ± 18 nM, $P < 0.05$), reduced rate of generation (46 ± 4 vs. 26 ± 5 nM min⁻¹, $P < 0.05$), and prolonged time to peak (11 ± 1 vs. 13 ± 1 min, $P < 0.05$). Overall, there was a modest but significant inverse correlation between degree of fibrosis and peak thrombin generation ($r = -0.4$, $P < 0.05$).

Conclusion: In a cohort of well compensated cirrhotic patients of mixed aetiology, thrombin generation is impaired as liver disease progresses before PT becomes prolonged. This may act as a marker of future decompensation or serve as a future therapeutic target.

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OR219

Early von Willebrand factor degradation upon acute exposure to high shear stress under continuous-flow circulatory assist devices

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Background: Left Ventricular Assist Devices (LVAD) are associated with a high rate of early surgical bleedings. An acquired deficiency of von Willebrand factor (VWF), characterized by a loss of high molecular weight multimers (HMWM) was reported within days following implantation of LVADs. *In-vitro* studies inferred that unfolding and cleavage of VWF *in-vivo* could occur within 200 s under high shear conditions. We hypothesized that VWF degradation could occur within minutes at initiation of LVAD-therapy, when blood is instantaneously exposed to high shear stress.

Aims: To investigate the time course of HMWM defect under LVAD and the potential underlying mechanisms.

Methods: Anticoagulated human blood from healthy donors was perfused in a HeartMate-II® (HM-II) mock circulatory system. A time-course of VWF analysis was performed setting the rotor speed to 9000 rpm ($n = 10$), as in HM-II-patients, and to 3000, 6000 and 12,000 rpm ($n = 3$ each). Three more runs were performed at 9000 rpm with EDTA. A similar time-course analysis was performed in 8 HM-II patients at initiation of LVAD-support. Samples were collected before (T0) and after initiation of HM-II® support (T5, T30 and T180 min) for VWF antigen, propeptide (VWFpp), proteolytic fragments, multimeric analysis, and PFA-CADP.

Results: A time-dependent loss of HMWM was observed in LVAD-patients ($P < 0.01$) along with an acute increase in VWFpp, already significant at 5 min ($P = 0.01$). PFA-CADP was also significantly increased 5 min after initiation of LVAD-support ($P = 0.01$). HMWM loss was more pronounced *in-vitro* than in LVAD-patients and was complete after 180 min ($P < 0.0001$). VWF degradation *in-vitro* was dependent from LVAD-speed ($12,000 > 9000 > 6000$ rpm) and inhibited when spiking EDTA before pump start. A time-dependent increase in VWF proteolytic fragments was observed in LVAD-patients.

Conclusion: An acquired VWF deficiency, mainly related to an excessive VWF proteolysis, occurs at initiation of LVAD-therapy, a condition prone to early surgical bleedings.

Disclosure of Interest: None declared.

OR220

Hemostatic disorder of uremia: patients with terminal chronic renal failure (CRF) have increased platelet tissue factor derived intravascular clotting activation with delayed clot lysis time in platelet rich plasma (CLT-PRP)

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Background: Co-existence of circulating markers of hemostatic activation, inflammation, and oxidative stress in CRF configures a picture of mild, protracted, subclinical DIC. These processes are risk factors for atherothrombosis, major complication of terminal CRF.

Aims: To study the platelets role in the pathogenesis of the hemostatic defect of CRF, by testing a global fibrinolytic assay (CLT-PRP) in patients with CRF on peritoneal dialysis (CRF-PD).

Methods: We studied 22 age, sex-matched CRF-PD patients and controls. We measured platelet aggregation (LPA) and 5-HT secretion (PS) with sub-threshold agonist concentrations, CLT-PRP (Panes et al. Platelets 2012; 23:36), plasma D-dimer, tPA, PAI-1, platelet Tissue Factor-dependent procoagulant activity (TF-PCA), thrombin generation in PRP (TG), F1 + 2, us-PCR, TNF α , advanced oxidation protein products (AOPP) and VWF:Ag.

Results: Compared with Controls, patients with CRF-PD had enhanced TF-PCA (44 vs. 20.8 nM FXa $\times 2 \times 10^{-7}$ platelets, $P = 0.009$) and delayed normalized CLT-PRP (2.6 ± 0.8 vs. 1.4 ± 0.5 , $P < 0.0001$, expressed as time patient/time standard PPP). Interestingly, CLT-PRP and TF-PCA were not correlated in patients whereas in Controls they showed a strong negative association ($r = -0.80$, $P < 0.0001$). Patients had higher plasma F1 + 2 and D-Dimers than Controls ($P < 0.05$), and these were correlated with CLT-PRP ($P < 0.005$). Significantly higher us-PCR, TNFa, VWF:Ag, and AOPP, but lower tPA and velocity index of TG-PRP were found in patients than in Controls. LTA, PS and plasma PAI-1 did not differ in both groups.

Conclusion: Patients with CRF-PD course with mild inflammation, increased oxidative damage, endothelial dysfunction, and activated clotting. Increased D-dimers reflect better fibrin generation than activated fibrinolysis. Our results denote a disturbed hemostatic balance with an exacerbated platelet-derived TF-PCA and a delayed fibrin lysis in CRF-PD. Globally, these findings may be prominent pathogenetic factors in the atherothrombotic complications of CRF. (FONDECYT 1030853).

Disclosure of Interest: None declared.

Pediatric hemostasis

OR221

Role of the G proteins and SYNTAXIN-11 in the differences of platelet reactivity and secretion between neonates and adults

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Background: Compared to adult platelets (APs), neonatal platelets (NPs) are hyporeactive in response to most agonists and show impaired granule release despite number and content of NPs and APs granules are similar. The molecular bases of this hyporeactivity are not completely known.

Aims: To compare the expression levels of (i) G proteins, crucial transducers of platelet reactivity and (ii) elements of the fusogenic complex, essential for platelet secretion, between NPs and APs.

Methods: Platelets from umbilical cord or adult peripheral blood ($n \geq 12$ /group) were purified by filtration and magnetic cellular separation from platelet-rich plasma. *Gαq*, *Gαi2*, *Gαs*, and *Gαz*, SNAP23, VAMP8, SYNTAXIN-11 (STX11), and MUNC18b mRNA and protein levels were quantified by qRT-PCR and western blot, respectively.

Results: NPs showed significant lower *Gαq* (40%) and *Gαz* (60%) and higher *Gαs* (40%) and *Gαi2* (70%) mRNA levels than APs. Accordingly, we detected higher *Gαs* (30%) and *Gαi2* (25%) and significant lower *Gαq* (20%) and *Gαz* (30%) protein levels. Regarding the SNARE proteins, NPs in comparison with APs, had ~30% ($P = 0.01$) and ~25% ($P < 0.05$) decrease in STX11 and Munc18b protein levels, respectively, and a 3-fold reduction in *STX11* mRNA levels ($P < 0.01$). We did not find differences in the other SNARE proteins.

Conclusion: We show that NPs and APs exhibit differences in the G proteins expression which likely contribute to NPs hyporeactivity. The underexpression of *Gαq* in NPs may influence to their poor response to TxA₂, ADP or thrombin and lower *Gαz* may promote the poor response to epinephrine. As *Gαs* increases and *Gαi2* inhibits cAMP synthesis, their simultaneous increase in NPs may constitute a mutually compensatory mechanism. We also show that NPs display lower STX11 and Munc18b protein levels than APs. Since STX11 performs an essential role in α and δ granule release reaction and Munc18b regulates its function, our findings provide new elements to explain differences in platelet secretion and reactivity between neonates and adults.

Disclosure of Interest: None declared.

OR222

Disseminated intravascular coagulation contributes an independent mortality risk in critically ill children

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Background: Disseminated intravascular coagulation (DIC) occurs frequently in gravely ill children. Clinical trials for DIC in the pediatric intensive care unit (PICU) have not shown improved survival and the role of DIC in mortality is unclear.

Aims: to determine mortality attributed to DIC in children in the PICU.

Methods: De-identified records were extracted on all children admitted to Children's Hospital Colorado PICU from January 1, 2011 through December 31, 2013 including pediatric risk of mortality score (PRISM), mortality, days of ventilator, vasopressor, or PICU support and blood products administered. ISTH DIC score (Taylor FB, *Thromb Haemost* 2001) was calculated for tests obtained within 48 h of admission.

Results: Three hundred and fifty-two (6.2%) of 5693 PICU patients had DIC testing within 48 h of admission. 294 (84%) tested had DIC score < 5 (negative or non-overt DIC), while 58 (16%) had DIC score ≥ 5 (overt DIC). Tested and non-tested children were similar in age, gender and ventilator days. Mortality, PRISM score, circulatory support and blood product usage were lower in untested children. DIC testing conveyed an increased risk of death (ROD) with an odds ratio (OR) of 7.37 (confidence intervals, (CI) 5.21, 10.4, $P < 0.0001$). DIC score as a continuous or categorical variable increased ROD (OR 2.11, CI 1.69, 2.63, $P < 0.0001$; OR 9.8 (CI 5.05, 19.03, $P < 0.0001$ respectively). When adjusted for PRISM score: 1) DIC testing no longer independently predicted death (OR 1.33, CI 0.77, 2.32, $P = 0.307$); 2) DIC score as a continuous variable approached significance for ROD (OR 1.31, CI 0.99, 1.73, $P = 0.058$); and 3) DIC score as a categorical variable (≥ 5) remained significant in predicting ROD (OR 2.6, CI 1.06, 6.35, $P = 0.036$).

Conclusion: Overt DIC using ISTH score conveys a modest independent risk of death for children in the PICU. PRISM score accounts for the major mortality risk of critically ill children and may explain why DIC treatment trials have not improved survival in this population.

Disclosure of Interest: None declared.

OR223

Impaired collagen response in neonatal platelets: the balance between activation and inhibition pathways

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Background: Platelet activation by collagen involves the glycoprotein VI-Fcγ receptor complex, and subsequent recruitment of phosphatidylinositol 3-kinase (PI3K) to the phosphorylated linker for activation of T cells (LAT)-based signalosome. This pathway is negatively regulated by phosphorylated platelet endothelial cell adhesion molecule-1 (PECAM-1), which inhibits the association of the PI3K-p85 subunit with the LAT signalosome. When compared to platelets from adult controls (AC), neonatal platelets from umbilical cord blood (UCB) show decreased collagen-induced LAT phosphorylation, calcium mobilization and aggregation.

Aims: To determine the contribution of PECAM-1 to the decreased collagen response in neonatal platelets.

Methods: Washed suspensions of UCB platelets and AC platelets, stirred in an aggregometer, were stimulated with collagen ($10 \mu\text{g mL}^{-1}$; 20–60 s), lysed and immuno-precipitated with specific antibodies to PECAM-1 or LAT, then immunoblotted with the precipitating antibody or anti-phosphotyrosine antibody, 4G10. Immunoblot band intensities were quantitated by scanning densitometry.

Results: Comparison of mean band intensities of LAT and PECAM-1 in immunoblots of platelet lysates showed no difference between UCB and AC platelets. Following stimulation, the ratio of phospho-LAT to total LAT was lower in UCB platelets [$\text{p-LAT/LAT} = 0.42 \pm 0.24$] than in AC platelets [$\text{p-LAT/LAT} = 0.82 \pm 0.2$; $P < 0.05$]. In contrast, the ratio of phospho-PECAM-1 to total PECAM-1 did not differ between UCB platelets [$\text{p-PECAM-1/PECAM-1} = 0.75 \pm 0.24$] and AC platelets [$\text{p-PECAM-1/PECAM-1} = 0.85 \pm 0.26$; n.s.].

Conclusion: Phosphorylation of PECAM-1 is comparable in UCB and AC platelets. These data are consistent with the impaired collagen acti-

vation in neonatal platelets resulting from a negative balance between the activation of LAT (decreased in UCB platelets) and inhibition of the signalosome assembly by activated PECAM-1 (not decreased in UCB platelets). The net effect is inhibition of downstream signaling and platelet activation.

Disclosure of Interest: None declared.

OR224

The contribution of pro- and anticoagulant processes to thrombin generation in the course of maturation

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Background: Coagulation factors undergo quantitative and qualitative changes with age and haemostatic tests show different results in children and adults. Thrombin generation (TG) is known to be decreased in children, but it is not yet known which processes mediate these age-specific differences in TG capacity.

Aims: Using a new approach to analyze the underlying processes of TG separately, we aimed to determine the contribution of pro- and anticoagulant processes to the overall effect on TG.

Methods: Plasma samples were collected from 44 healthy subjects in the range of 0.6–59.2 years of age. Antithrombin (AT), α_2 Macroglobulin (α_2 M), and prothrombin levels were determined. TG was measured at 1 and 5 pM tissue factor and the TG curves were split into prothrombin conversion and thrombin inactivation curves, using a validated algorithm.

Results: TG quantified as ETP was reduced in children (1095 ± 313 nM min vs. 1583 ± 352 nM min). Prothrombin levels were lower (0.90 ± 0.13 μ M vs. 1.09 ± 0.18 μ M; $P = 0.002$) and α_2 M levels were higher (5.28 ± 0.87 μ M vs. 3.48 ± 1.15 μ M; $P < 0.001$) in children than adults. In children, less prothrombin was converted during TG (734 ± 225 nM vs. 1038 ± 179 nM; $P = 0.001$), and the maximum rate of prothrombin conversion (i.e. prothrombinase activity) was reduced (204 ± 142 nM min⁻¹ vs. 282 ± 49 nM min⁻¹; $P = 0.001$). Less thrombin-AT complexes are formed during TG in children ($P < 0.001$). While the absolute amount of formed thrombin- α_2 M was comparable to adults, proportionally α_2 M plays a larger role in children. Further in silico experimentation showed that normalization of prothrombin conversion and α_2 M levels in children to adult levels increased TG to the level observed in adults.

Conclusion: Young age has a dual effect on thrombin generation: Prothrombin conversion is reduced due to lower plasma prothrombin levels and lower prothrombinase activity, and the balance between thrombin inhibitors AT and α_2 M shifts towards α_2 M in children.

Disclosure of Interest: R. Kremers Employee of: Synapse, R. Wagenvoort Employee of: Synapse, B. de Laat Employee of: Synapse, P. Monagle: None declared, C. Hemker Employee of: Synapse, V. Ignjatovic: None declared.

OR225

Pediatric reference values of thrombin generation measured by the Calibrated Automated Thrombogram (CAT)

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Background: Developmental haemostasis is characterized by deficiencies of pro- and anticoagulants in young children, suggesting a disturbed balance in coagulation. Thrombin generation assessed by the Calibrated Automated Thrombogram (CAT) reflects the overall capability of plasma to generate thrombin.

Aims: Aim: To use CAT in pediatric research in the field of haemostasis, we now determined thrombin generation reference values for healthy children and adults.

Methods: After informed consent citrated full blood samples were obtained from 199 children, age groups: 2–6 months ($n = 25$); 7–12 months ($n = 18$); 1–5 years ($n = 52$); 6–10 years ($n = 54$), 11–18 years ($n = 50$) and adults > 19 years ($n = 52$). The study was approved by the medical ethical committee of the Erasmus MC. Thrombin generation was assessed by means of the CAT method using 40 μ L platelet-poor plasma triggered with 1 pM tissue factor and 4 μ M phospholipids (final concentrations), with and without thrombomodulin.

Results: Peak height (112 ± 34 nM) and ETP (654 ± 177 nM min) were significantly lower in the youngest group compared to adults (peak height: 158 ± 72 nM, ETP: 1059 ± 331 nM min). Thrombin generation increased gradually from 2 to 6 months to adolescents of 11–18 years (Pearson correlation 0.97 for ETP). In plasma from adults, thrombomodulin addition induced a near normal reduction in ETP (35%), whereas attenuation of thrombin generation was reduced at younger age (2–6 months: 20%). Levels of protein C and S were approximately 30% lower at the age of 2–6 months compared to adults (own reference values JTH 2012;10:2254–63).

Conclusion: Lower ETP levels and less ETP reduction in the presence of thrombomodulin point towards diminished thrombin generation in the youngest children, most likely related to less effective inhibition of thrombin generation by the protein C pathway.

Disclosure of Interest: None declared.

Cancer and thrombosis – clinical – II

OR226

Longterm cancer risk after venous thromboembolism

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Background: A venous thromboembolic event (VTE) may indicate an underlying cancer. In population based studies the likelihood of cancer is highest during the first year but a persistent increase has been reported also in the years thereafter. Data on the risk in patients who completed anticoagulant treatment and recommendations regarding screening are lacking.

Aims: To evaluate the long-term risk of cancer in a well-defined cohort of patients with an unprovoked VTE.

Methods: We prospectively followed patients with a first unprovoked VTE after discontinuation of anticoagulation and excluded those with long-term antithrombotic therapy, major thrombophilia or pregnancy. Study endpoint was diagnosis of cancer. The ratio of the observed cases and the number of cases expected based on national cancer incidence rates was calculated and expressed as standardized incidence ratio (SIR). The study was approved by the local ethics committee and all patients consented.

Results: Forty-three (3.6%) of 1188 patients (mean age 48 years, 638 women, median duration of anticoagulation 7.2 months) were diagnosed with cancer (colon 8, breast 7, lung 6, hematologic 6, prostate 4, skin 1, brain 1, others 10) during a median follow up of 70 months (range 3–216). Cancer patients were significantly older (57 vs. 48 years, $P < 0.001$) but did not differ from non-cancer patients regarding proportion of women (49% vs. 54%), blood cell counts (hemoglobin 14.2 vs. 14.2 g dL⁻¹; platelets 232 vs. 238 G L⁻¹; leukocytes 6.5 vs. 6.8 G L⁻¹), body mass index (both 27 kg m⁻²) or D-Dimer (469 vs. 463 µg mL⁻¹). The probability of cancer was 0.7% (95% CI 0.1–1.3%), 1.7% (95% CI 0.9–2.5%) and 3.0% (95% CI 1.8–4.2%) after 1, 2 and 5 years. The corresponding SIR did not significantly differ from expected rates in an age- and sex-matched general population (1.2, 1.3 and 1.0).

Conclusion: In patients with unprovoked VTE the long-term incidence of cancer after anticoagulation is low. Cancer types are heterogeneous and specific screening strategies do not seem to be warranted.

Disclosure of Interest: None declared.

OR227

Hypercoagulability, venous thromboembolism and death in patients with cancer: a multi-state model

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Background: Venous thromboembolism (VTE) is a frequent complication in patients with cancer.

Aims: To study outcomes in cancer patients at risk of VTE using multi-state modeling.

Methods: We formulated a unidirectional illness-death model with 3 transitions (transition#1: alive without VTE to VTE, transition#2: alive without VTE to death without VTE, transition#3: VTE to death). The risks of all 3 transitions were analyzed with 'Semi-Markov' multi-state models.

Results: One thousand seven hundred and twenty-nine cancer patients with newly-diagnosed cancer or progressive disease after remission were followed for a median interval of 2 years. Of these, 125 patients (7.3%) developed VTE, 72 patients (57.6%) died after developing VTE, and 594 patients (34.4%) died without developing VTE, respectively. Incident VTE was associated with a more than threefold increase in the risk of transition to death (hazard ratio [HR] = 3.4, 95% CI: 2.6–4.3, $P < 0.001$). The adverse impact of VTE on survival was highly dependent on the time point of VTE occurrence. Whereas a VTE event at 3 months after baseline increased the risk of death by a factor of 3.2 (HR = 3.2, 95% CI: 2.5–4.2, $P < 0.001$), a VTE event after 1 year was associated with a more than 5-fold increase in the risk of death (HR = 5.4, 95% CI: 3.2–8.5, $P < 0.001$). Elevated baseline D-Dimer levels were associated with higher transition risks towards VTE (HR per 1 µg mL⁻¹ increase = 1.07, 95% CI: 1.04–1.10, $P < 0.001$), death without VTE (HR = 1.07, 95% CI: 1.05–1.08, $P < 0.001$), and death after VTE (HR = 1.17, 95% CI: 1.11–1.22, $P < 0.001$). This finding prevailed after adjusting for tumor type, and implies that patients with elevated D-Dimer not only have a higher risk of VTE and death, but also die faster after developing VTE than patients with VTE and lower baseline D-Dimer levels.

Conclusion: Multi-state models provide a statistically efficient representation of the clinical course of cancer patients at risk of VTE, and yield insights into the disease process beyond what can be observed with single-endpoint analyses.

Disclosure of Interest: None declared.

OR228

Characteristics and risk factors of major and clinically relevant non-major bleeding in cancer patients receiving anticoagulant treatment for acute venous thromboembolism – the CATCH study

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Background: Cancer patients with acute venous thromboembolism (VTE) receiving anticoagulant treatment have a substantial risk of bleeding complications.

Aims: To assess the rate, site and risk factors of clinically relevant bleeding (CRB; major or clinically relevant non-major bleeding) in cancer patients receiving anticoagulation for VTE.

Methods: CATCH is a randomized, open-label, multicenter, Phase III trial (NCT01130025) comparing tinzaparin 175 IU kg⁻¹ once daily for 6 months vs. initial tinzaparin 175 IU kg⁻¹ once daily for 5–10 days and warfarin (target INR 2.0–3.0) for 6 months in patients with active cancer and acute, symptomatic VTE. Tinzaparin dose was not reduced for renal impairment (creatinine clearance [CrCl] < 60 mL min⁻¹). All patients were followed for bleeding until 24 h after the last dose of anticoagulant up to 6 months. Blinded central adjudication was performed for all bleeding events.

Results: Among 900 randomized patients, 145 (16%) patients had 189 CRBs and 42 (4.7%) died within 30 days after CRB. Common bleeding sites were gastrointestinal (38%), genitourinary (22%), nose (10%) and intracranial (2%). In univariate analysis, risk of CRB was increased with metastatic disease (relative risk [RR] 1.62; 95% CI 1.14–2.31); age > 75 years (RR 1.78; 95% CI 1.20–2.66); and 1° or 2° intracranial malignancy (RR 1.99; 95% CI 1.13–3.49). Of the 29 patients with intracranial lesions, 9 (31%) had a CRB, of which 1 was intracranial. In the tinzaparin group, bleeding risk was not increased in patients with CrCl < 60 mL min⁻¹ ($n = 67$), compared with those with CrCl ≥ 60 mL min⁻¹ ($n = 355$; RR 1.27; 95% CI 0.69–2.32). In the warfarin group, INR was a poor predictor of bleeding, with 42% of CRBs occurring with an INR ≤ 3.0.

Conclusion: CRB occurs in 16% of cancer patients with symptomatic VTE during anticoagulant treatment and is associated with metastatic disease, older age and intracranial lesions. Treatment with full-dose tinzaparin seems safe in patients with renal impairment.

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OR229

Thrombin generation and venous thromboembolism among multiple myeloma patients receiving lenalidomide and prednisolone maintenance following autologous stem cell transplantation

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Background: Lenalidomide, used in the treatment of multiple myeloma (MM) is associated with an increased risk of venous thromboembolism (VTE). Thrombin generation (TG) might be a useful surrogate marker of thrombosis risk.

Aims: To sequentially monitor TG in MM patients receiving lenalidomide and alternate day prednisolone (RAP) following ASCT and correlating it with objectively confirmed VTE.

Methods: The LEOPARD study was a phase II, open label, single arm, multi-centre study of RAP maintenance therapy commencing 6–8 weeks after a single MEL200 ASCT for patients with MM. RAP was continued until toxicity or relapse/progression. Plasma samples of participants were collected and frozen pre-ASCT, post-ASCT prior to commencing RAP maintenance and at six months post ASCT. TG was measured using calibrated automated thrombography (CATTM) blinded to symptomatically confirmed VTE following completion of the study.

Results: Twenty-eight of 60 study participants had available all three predetermined plasma samples for analysis and remained on RAP at 6 m post ASCT. Female = 14, male = 14 with a median age of 61 years (range, 41–71). 44 and 6 patients received aspirin and enoxaparin post ASCT respectively. 3 out of 60 patients (5%) had objectively confirmed symptomatic VTE.

Endogenous thrombin generation (mean ETP) did not differ significantly between pre-ASCT (1682 nM), post-ASCT (pre RAP maintenance, 1645 nM) and 6 months post ASCT (1537 nM). Similarly, there was no significant difference between peak thrombin (mean PTG) at each of the time points, 376 nM, 388 nM and 367 nM respectively. No apparent difference in TG between the 3 patients with VTE and the remainder of the cohort was evident at pre RAP maintenance & 6 months post ASCT (3 VTE patients mean ETP, 1396.8 nM and 1385.8 nM; mean peak PTG 216.5 nM and 221.7 nM).

Conclusion: The risk for VTE was low in this cohort of MM patients receiving maintenance RAP following ASCT, with no significant difference in TG (both ETP and PTG) at pre-ASCT, post-ASCT (pre RAP maintenance) and 6 months post ASCT.

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OR230

Incidental venous thromboembolism at thoracoabdominal staging CT in colorectal cancer

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Background: Venous thromboembolism (VTE) is a well-recognized cause of mortality in patients with colorectal cancer.

Aims: To analyse 1) the prevalence and natural history of incidental VTE detected at primary thoracoabdominal staging CT scans in patients with colorectal cancer (CRC), and 2) clinicopathological factors associated with VTE.

Methods: All primary staging CT scans with intravenous contrast performed at our Center between 2006 and 2011 were reviewed by an expert radiologist for manifest VTE. Potential confounders were obtained from the Danish Colorectal Cancer Group database, the National Patient and Pathology Registries. Multivariable logistic- and extended Cox-regression analyses were used to adjust for confounders.

Results: In 810 of 1287 patients assessed for inclusion, a staging CT scan and data on clinicopathological factors were available. Twenty-three patients (2.8%) had a manifest VTE on the scan. Pulmonary, splanchnic and iliofemoral VTE were detected in 15 (1.9%), 6 (0.7%) and 4 (0.5%) patients, respectively. Two patients had VTE at multiple sites. VTE was associated with synchronous metastatic disease (SMD), adjusted odds ratio 2.70 (95% confidence interval (CI):1.15–6.27, $P = 0.020$), but not statistically associated with gender, age, index tumour location or comorbidity. VTE was associated with an impaired 30-day survival (adjusted Hazard Rate [aHR] = 7.20, 95% CI:2.04–25.47, $P = 0.002$), but had no significant impact on long-term survival beyond 30 days (aHR = 1.56, 95% CI:0.79–3.09).

Conclusion: Incidental VTE can be detected at routine thoracoabdominal CT scan in the primary staging of nearly 3% of CRC patients and is associated with SMD. The radiologist should endeavour to detect VTE, as they have major impact on 30-day survival.

Disclosure of Interest: None declared.

Megakaryocytes and Thrombopoiesis II

OR231

Syndromes with gray platelets: *NBEAL2* expression is regulated by transcription factor *GATA1*

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Background: Gray Platelet Syndrome (GPS) is named after the gray appearance of platelets due to absence of α -granules. GPS is caused by recessive mutations in the *NBEAL2* gene, resulting in macrothrombocytopenia and myelofibrosis. Gray platelets have also been described due to X-linked *GATA1* and dominant *GFI1B* mutations. We identified mutations in *NBEAL2*, *GATA1* (Freson, Blood, 2001; HMG, 2002) and *GFI1B* (Chen, Science, 2014) in patients with enlarged platelets with paucity of α -granules.

Aims: Because the patients have similar platelet phenotypes, we hypothesize that *GATA1* and *GFI1B* regulate *NBEAL2* expression, which is important for α -granule formation.

Methods: Immunoblots were performed on patients' platelet extracts. ChIP-sequencing revealed 3 *GATA* binding sites (BS1, BS2, BS3) in a regulatory region 31 kb upstream of *NBEAL2* (Tijssen, Dev Cell, 2011). This region is a predicted long-range enhancer for the *NBEAL2* promoter. By cloning this region into a luciferase reporter construct and measuring the luciferase activity we studied the regulatory activity of this region. Similar expression studies were obtained after mutagenesis of the 3 *GATA*-bindingsites. Binding of *GATA1* to *GFI1B* was studied with a GST pull-down assay.

Results: GATA1, but not GFI1B deficient platelets expressed very low levels of NBEAL2 protein. We showed that the regulatory region with GATA binding sites is a strong enhancer that further increases expression upon GATA1 overexpression. Mutagenesis of BS1 resulted in higher promoter activity while mutagenesis of BS2 or BS3 resulted in lower activity compared to the wild type sequence. We showed a direct interaction between GATA1 and GFI1B. Further studies will reveal if this interaction is essential for *NBEAL2* expression.

Conclusion: Our data confirm that the regulatory region upstream of the *NBEAL2* promoter acts as an enhancer via binding of GATA1. Additional experiments are being performed to elucidate the exact mechanism by which GATA1 stimulates *NBEAL2* expression, potentially via GFI1B.

Disclosure of Interest: None declared.

OR232

Dynein-dependent microtubule sliding drives proplatelet elongation

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Background: Blood platelets are derived from bone marrow megakaryocytes (MKs). MKs extend long cytoplasmic protrusions (proplatelets) into the blood vessel, from which platelet intermediates and platelets are sequentially released.

Aims: While microtubule forces are known to be essential for proplatelet and platelet production, details on how they interact to extend proplatelets and release platelets are not defined.

Methods: We used chemicals that interfere with different aspects of microtubule function and directly visualized microtubule movements using two quantitative fluorescence techniques: fluorescence recovery after photobleaching (FRAP) and fluorescence loss after photoconversion (FLAC). Finally, we capitalized on the recent development of a microfluidic platelet bioreactor to study microtubule movements under physiological shear stress and compared this to MKs in static culture.

Results: We show that proplatelet formation is a process that can be divided into repetitive phases of motility: extension, pause and retraction. We quantified proplatelet elongation with time in static MK culture conditions and determined that proplatelets move in bursts and the fastest unidirectional rates varied from 4 to 13 $\mu\text{m min}^{-1}$. However, incorporation of the pause time reduced the rate of elongation to an average rate of $\sim 0.85 \mu\text{m min}^{-1}$. Proplatelets formed by MKs within the platelet bioreactor elongated at an average rate of 30 $\mu\text{m min}^{-1}$ which is much higher than the rate measured for proplatelet formation in culture, and more accurately reproduces proplatelet extension rates *in vivo*. Furthermore we demonstrate that not tubulin polymerization but microtubule sliding drives proplatelet elongation and is dependent on cytoplasmic dynein under static condition and physiological shear stress by using FRAP in proplatelets with fluorescence-tagged β 1-tubulin.

Conclusion: A refined understanding of the specific mechanisms regulating platelet production will yield strategies to treat patients with thrombocytopenia or thrombocythemia.

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financial interest in Platelet BioGenesis. J.E.I. is inventor on this patent. J. Hartwig: None declared.

OR233

B4galactosyltransferase 1 (B4GalT1) is a key regulator of hematopoietic stem cell function and thrombopoiesis

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Background: Delayed platelet recovery following hematopoietic stem cell (HSC) transplantation is associated with decreased survival. New strategies may reduce the need for blood products and decrease the risks associated with platelet transfusions. Glycans are key regulators of platelet survival, specifically lactosaminoglycan (LacNAc or β Gal1,4 GlcNAc), but their role in thrombopoiesis is understudied.

Aims: Here we investigated the role of LacNAc structures, synthesized by the galactosyltransferase β 4GalT1 through the addition of galactose (Gal) to terminal N-acetylglucosamine (GlcNAc), in the regulation of HSC function and thrombopoiesis.

Methods: Fetal liver cells (FLCs, E14.5) and bone marrow cells (BMCs, 8 weeks) were transplanted into lethally irradiated WT mice using non-competitive and competitive (10:1 ratio) approaches. Complete blood counts were obtained before and 1, 2, 3 and 4 weeks after transplant.

Results: Only 9% of β 4GalT1^{-/-} mice survived until adulthood. β 4GalT1^{-/-} fetuses (E14.5) and adult mice had thrombocytopenia (25% of normal), but increased white blood cells counts (by 115%). β 4GalT1^{-/-} mice had increased bone marrow megakaryocyte (MK) numbers, but $\sim 50\%$ of the β 4GalT1^{-/-} MKs failed to localize at sinusoids. *In vitro* cultured MKs had highly impaired proplatelet production capacity (10%). We next investigated the capacity of β 4GalT1^{-/-} HSC to restore hematopoiesis in lethally irradiated mice. β 4GalT1^{-/-} FLCs had normal, whereas BMCs had increased (by 40%) *in vitro* colony forming capacity. However, β 4GalT1^{-/-} FLCs and BMCs were unable to restore hematopoiesis when non-competitively transplanted into lethally irradiated WT mice, severely decreasing mouse survival (< 2 weeks). Competitive β 4GalT1^{-/-} HSC transplants restored normal white and red blood cells numbers, but not platelets, despite the presence of β 4GalT1^{-/-} MKs in the bone marrow.

Conclusion: β 4GalT1 activity is a key player of hematopoiesis and thrombopoiesis, specifically restoring platelet recovery post HSC transplantation.

Disclosure of Interest: None declared.

OR234

Type 2B von Willebrand disease (VWD) is characterized by a cytoskeletal desorganization in megakaryocytes leading to macrothrombocytopenia

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Background: Type 2B VWD patients display macrothrombocytopenia and defective platelet formation *in vitro* from megakaryocytes (MKs). However, the underlying mechanisms remain unknown.

Aims: To determine the molecular mechanisms in the type 2B VWD leading to macrothrombocytopenia.

Methods: We generated a knock-in mouse expressing the von Willebrand factor (VWF) p.V1316M mutation (2B mice).

Results: We first investigated MK differentiation in bone marrow. MK numbers, MK progenitor differentiation and ploidy were identical in wild type (WT) and 2B mice. Strikingly, 2B MKs spread over fibrinogen displayed decreased number of stress fibers with disorganized actin filaments that localized in a thick peripheral zone from which α -granules were excluded. Interestingly, platelet recovery following antibody-mediated depletion *in vivo* was slower (30% of control) in 2B mice, suggesting abnormal platelet kinetics. Proplatelet formation in mouse bone marrow samples and *in vitro* was drastically decreased in 2B mice (80% inhibition). Also, in a flow model at high shear rate, proplatelet formation and platelet release were impaired. Proplatelet formation is associated with cytoskeletal reorganization, and LIM kinases are known to control the dynamic assembly of actin and microtubules. Therefore, LIMK were studied and a 4-fold increase in phosphorylated LIMK1/2 in 2B MKs was seen. The roles of LIMK are the phosphorylation of cofilin, an actin turnover-regulating protein and the regulation of microtubule stability. 2B MKs displayed a 2.5-fold increase in the amount of the phosphorylated (inactive) form of cofilin, thus keeping actin in polymeric states, and in acetylated α -tubulin, a post-translational modification associated with microtubule stabilization.

Conclusion: Together, these data are consistent with an hyperactivation of LIMK in 2B MK leading to alteration of actin cytoskeleton regulation, potentially explaining the defect in proplatelet generation. The link between VWF2B and hyperactivation of LIMK remains to be elucidated.

Disclosure of Interest: None declared.

OR235

Receptor-mediated endocytosis is required for normal megakaryocyte demarcation membrane system formation and platelet production

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Background: Blood platelets are produced in the bone marrow by megakaryocytes (MKs) in a process that requires extensive intracellular membrane rearrangements. These include the formation of the demarcation membrane system (DMS), the surface-connected membrane extension that provides membranes necessary for future platelets. *Dnm2*^{f/f} *Pf4-Cre* mice specifically lacking the housekeeping GTPase dynamin 2 (DNM2) in MKs develop severe macrothrombocytopenia, MK hyperplasia, splenomegaly and myelofibrosis, due to impaired receptor-mediated endocytosis (RME) in MKs. Specifically, *Dnm2*^{f/f} *Pf4-Cre* MKs accumulate 'clathrin-coated profiles' that obstruct DMS formation *in vivo*, demonstrating that RME is required for normal MK development.

Aims: Here we investigated the role of the F-BAR protein PACSIN2, a DNM2 partner involved in caveolae-dependent RME, in DMS formation.

Methods: High-resolution structured illumination microscopy revealed PACSIN2 as an internal component of a well-defined invagination that characterizes the initiating DMS in MKs and of caveolae-like invaginations in platelets.

Results: *Pacsin2*^{-/-} mice developed mild thrombocytopenia and the DMS appeared less well defined and platelet territories were not readily visualized in *Pacsin2*^{-/-} MKs. *Pacsin2*^{-/-} platelets had abnormal morphology, with slightly enlarged volume and shallow shape. To

determine the contribution of PACSIN2 to the MK phenotype of *Dnm2*^{f/f} *Pf4-Cre* mice, *Pacsin2*^{-/-} *Dnm2*^{f/f} *Pf4-Cre* mice lacking both PACSIN2 and DNM2 in MKs were generated. Strikingly, additional PACSIN2 deletion rescued the severe thrombocytopenia, MK hyperplasia, myelofibrosis and splenomegaly of *Dnm2*^{f/f} *Pf4-Cre* mice. Specifically, PACSIN2 deletion abrogated the accumulation of 'clathrin-coated profiles', which were replaced by elongated membrane tubules in *Pacsin2*^{-/-} *Dnm2*^{f/f} *Pf4-Cre* MKs.

Conclusion: DNM2 and PACSIN2 regulate clathrin- and caveolae-dependent RME in MKs in concert, thereby playing a critical role in DMS formation and platelet production.

Disclosure of Interest: None declared.

Tissue factor and factor VII – II

OR236

Genome-wide serine protease knockdowns in zebrafish: identification of prostaticin in factor VII activation pathway

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Background: Human factor VII (f7) is a vitamin K dependent serine protease zymogen that plays a pivotal role in the initiation of coagulation. Its activated form, factor VIIa (f7a), circulates at 1% of the levels of total f7. The mechanism of the initial generation of f7a has been the subject of controversy and continues to be elusive.

Aims: Our goal is to identify protease/s involved in the activation of f7.

Methods: We used a piggyback knockdown method, kinetic Prothrombin Time (kPT) assay and f7 and f7a ELISA assays.

Results: We hypothesized that a knockdown of all proteases encoded by the genome and subsequent estimation of f7a levels in zebrafish plasma may identify any protease involved in f7 activation. As an initial step we performed piggyback knockdowns of 181 serine protease genes in adult zebrafish. We then screened their plasma with kPT assay to select only those genes involved in the extrinsic pathway of coagulation, which includes f7 gene. Knockdowns that yielded a prolonged kPT were then tested for a reduction in f7a levels by the f7a assay. In primary screens we found knockdown of 22 genes yielded prolonged kPT. Secondary and tertiary screens confirmed 15 genes to have a prolonged kPT. These 15 genes were then subjected to knockdowns again and then the plasmas were screened for f7a levels in order to elucidate their role in f7 activation. Knockdown of 6 genes *f2*, *f7*, *f10*, *hpn*, *prostaticin*, *hgfl* resulted in lower f7a levels. However, knockdowns of *f7* and *hgfl* have yielded lower f7 levels compared to f7 levels in *prostaticin* knockdowns.

Conclusion: Taken together, these results suggested that prostaticin participates in f7a generation rather than controlling f7 levels. From the literature, we found matriptase activates prostaticin and prostaticin activates hepsin. Interestingly, hepsin has also been shown to activate prostaticin. We have shown hepsin activates f7 in zebrafish. Thus, we believe a membrane bound serine protease cascade may be responsible for circulating f7a levels in zebrafish plasma.

Disclosure of Interest: None declared.

OR237

Tissue-factor induced allosteric enhancement of factor VIIa activity by stabilization of segment 215–219 and taming of W215 flexibility

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Background: The complex of coagulation factor VIIa (FVIIa) and tissue factor (TF) initiates blood coagulation upon vascular injury. Previous work has shown that TF binding to FVIIa generates new exosites for macromolecular substrates and induces allosteric changes in and around the FVIIa active-site, leading to enhanced FVIIa catalytic efficiency. Structural studies have added detailed molecular insights into the TF binding epitope, but have not elucidated the extensive and long-range structural effects in FVIIa.

Aims: To understand the molecular basis of TF-mediated allosteric regulation of FVIIa activity.

Methods: In the current study we have produced 3 FVIIa variants in which the 170-loop has been replaced with the corresponding loop from trypsin. The resulting variants have been investigated using a combined approach of functional and biophysical characterization, x-ray crystallography and molecular dynamics (MD) simulations.

Results: The functional characterization and x-ray crystal structures of these FVIIa variants suggest that stabilization of the segment 215–219 leads to maturation of the primary specificity pocket and enhanced catalytic efficiency, independent of protease domain N-terminus insertion. MD simulations support the experimental results and vouch for the direct involvement of W215 in TF-induced allosteric changes in the FVIIa protease domain. Our results are further corroborated by novel tryptophan quenching studies and solvent accessible surface area calculations.

Conclusion: We propose that in the absence of TF, FVIIa exists in a conformation where segment 215–219 is highly flexible, capable of occupying either a collapsed or an open conformation, functioning as an activity regulating mechanism. In addition to other changes, the binding of TF to FVIIa stabilizes segment 215–219 in an open conformation leading to enhanced functional properties of FVIIa. Taken together, our results shed new insights into molecular mechanism of TF-induced allosteric enhancement in FVIIa activity.

Disclosure of Interest: A. Sorensen Employee of: Novo Nordisk A/S, J. Madsen: None declared, A. Svensson Employee of: Novo Nordisk A/S, H. Stennicke Employee of: Novo Nordisk A/S, H. Østergaard Employee of: Novo Nordisk A/S, M. Overgaard: None declared, O. Olsen Employee of: Novo Nordisk A/S, P. Gandhi Employee of: Novo Nordisk A/S.

OR238

Relationships of circulating coagulation factor VIIa (FVIIa) with common single nucleotide polymorphisms and risk of incident ischemic stroke: the Cardiovascular Health Study

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Background: A small but variable fraction (~1%) of coagulation factor VII circulates as an active protease (FVIIa) in human plasma. Data

for FVIIc, (influenced by both FVIIa and total FVII), as a cardiovascular disease (CVD) risk factor has been inconsistent. To date, no studies have evaluated the genetic and epidemiologic associations of FVIIa.

Aims: Evaluate the associations of FVIIa with common single nucleotide polymorphisms (SNPs) and incident CVD events in a prospective population-based cohort of older adults.

Methods: FVIIa was measured by commercial clot-rate assay (Stago) in citrated plasma of ~3500 men and women ≥ 65-years from the Cardiovascular Health Study. Associations of FVIIa with ~50K SNPs were evaluated using the IBCv2 genotyping array. Cox proportional hazards models were used to calculate associations with incident CVD outcomes (median follow-up 14.5 years).

Results: FVIIa was correlated with FVIIc (Pearson $r^2 = 0.44$; $P < 0.0001$). Mean FVIIa was higher in women than men [57.9 vs. 45.9 mU mL⁻¹] ($P < 0.0001$) and in European-Americans (EAs) than African-Americans ($P < 0.05$). In EAs, 15 SNPs located in the *F7/F10* locus (chromosome 13) and 7 SNPs located in the *PROCR* locus (chromosome 20) were significantly associated with FVIIa. The top *F7* SNP, rs6046 ($P < 4.9 \times 10^{-24}$), was associated with 23.8 mU mL⁻¹ lower FVIIa per minor allele; the top *PROCR* SNP, rs867186 ($P < 1.7 \times 10^{-12}$), was associated with 7.5 mU mL⁻¹ higher FVIIa per minor allele. After adjustment for CVD-risk factors, FVIIa was associated with incident ischemic stroke ($P = 0.03$). Those in the highest FVIIa tertile [≥ 61 mU mL⁻¹] had a 29% increased risk vs. those in the lowest tertile [< 42 mU mL⁻¹]. There were no significant associations of FVIIa with incident MI, CHD, or CVD-related mortality.

Conclusion: These results support the importance of the *F7/F10* and *PROCR* loci on variation in circulating FVIIa, and suggest FVIIa may be a risk factor for ischemic stroke in older adults.

Disclosure of Interest: None declared.

OR239

Epidermal tissue factor in wound healing

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Background: Healing of skin wounds relies on several biological processes, including hemostasis, inflammation, cell proliferation and tissue remodeling. It has been hypothesized that tissue factor (TF), an initiator of coagulation and regulator of inflammation, plays a role in the re-epithelialization of skin wounds. To investigate this function, wound healing was studied in Keratin-14 Cre+ TF^{fl/fl} mice, which do not produce TF in their keratinocytes.

Aims: To confirm that a lack of epidermal tissue factor influenced wound healing and to explore the mechanisms underlying this defect.

Methods: Full thickness dermal wounds were placed on the backs of K14 Cre+ TF^{fl/fl} mice and Cre- littermate controls using a 3-mm biopsy punch. The wound areas and number of healed mice were observed daily. Wound samples were collected during the course of healing for histological analyses and primary keratinocyte cultures were used to study signaling.

Results: Cre+ mice exhibited deletion of TF in the epidermis and the leading edge of healing wounds but maintained TF expression around blood vessels. Cre+ mice had larger wounds and slower healing times than Cre- mice and were more prone to bleeding.

Conclusion: Epidermal tissue factor is required for normal wound healing and influences bleeding, wound size and healing time. Further studies will investigate the cellular players underlying this phenotype.

Disclosure of Interest: None declared.

Platelets – Signal transduction II

OR241

Glutamate receptor interacting protein 1 mediates platelet adhesion and thrombus formation

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Background: Platelets and neurons share many molecules and proteins that are best described in neurons but also have a role in platelet function. These include granule trafficking proteins, serotonin and serotonin receptors, and glutamate receptors. We have discovered that platelets express proteins associated with glutamate receptor trafficking in neurons, but have a different role in platelets. This includes Glutamate Receptor Interacting Protein 1 (GRIP1).

Aims: Our study describes an important role for GRIP1 in platelet adhesion.

Methods: Platelet-specific GRIP1^{-/-} mice were created to elucidate GRIP1 function. Tail bleeding time and mesenteric thrombosis assays were performed to determine hemostatic and thrombotic function. Platelets were stimulated *in vitro* with platelet agonists (thrombin, 2-meADP) and granule release and platelet activation was measured by determining surface expression of CD62P and activated GPIIb/IIIa as well as PF4 and ATP release by ELISA. Adhesion behavior was determined by vessel platelet rolling experiments. Protein interactions were determined by mass spectrometry and confirmed with immunoprecipitation and Western blots.

Results: Platelet-specific GRIP1^{-/-} mice have normal platelet counts but prolonged bleeding times and delayed vessel thrombosis. WT and GRIP1^{-/-} platelets show no difference in agonist induced granule exocytosis or GPIIb/IIIa activation. However, *in vivo* platelet rolling velocity is significantly increased in GRIP1^{-/-} platelets indicating a platelet adhesion defect. Potential GRIP1 interacting proteins were identified and revealed GRIP1 binding with the GP1b-IX complex. Further experiments have confirmed that GRIP1 interacts with GP1b α , GP1b β , and 14-3-3. GRIP1^{-/-} platelets have altered GP1b-IX complex and 14-3-3 interactions in resting and activated conditions that may lead to reduced platelet adhesion.

Conclusion: We have found that GRIP1 is part of the GP1b-IX complex and may have an important role in platelet adhesion *in vivo*.

Disclosure of Interest: None declared.

OR242

Unexpected role of platelets in lung development depending on a platelet activation receptor, CLEC-2

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Background: We have previously identified CLEC-2 as a receptor for platelet-activating snake venom rhodocytin and its endogenous ligand Podoplanin. In recent years, we and other groups have revealed that CLEC-2 on platelets has a lot of physiological functions other than thrombosis and hemostasis, such as blood-lymphatic vessel separation, inflammation and cancer metastasis. We have previously reported that CLEC-2 null mice die shortly after birth. Therefore, we hypothesized that CLEC-2 on platelets also plays important roles in lung development.

Aims: The aim of this study is to elucidate CLEC-2 functions during lung development.

Methods: We conducted histological phenotypic analysis of the lung of CLEC-2 null mice and platelet-specific CLEC-2-deficient mice (CLEC-2 CKO, PF4-Cre CLEC-2^{fllox/fllox}) during developmental stages. We also injected anti-CLEC-2 antibody to pregnant female mice of CLEC-2 CKO or generated PF4-Cre CLEC-2^{fllox/-} fetus to completely deplete residual CLEC-2 expression from platelets and histologically analyzed the lung of the fetus.

Results: We found that CLEC-2 null mice showed alveolar septum malformation (thickened alveolar septum and narrow air spaces) which may be caused by abnormal distribution of myofibroblasts. To elucidate whether CLEC-2 on platelets are involved in lung development, we analyzed CLEC-2 CKO mice. The CKO mice showed similar phenotypes to CLEC-2 null mice, but the phenotypes are milder and they are viable. Because we found that CLEC-2 CKO fetal platelets showed small residual expression of CLEC-2, we attempted the further reduction of CLEC-2 expression in CLEC-2 CKO fetal platelets by genetic procedure and antibody administration. Intriguingly, these procedures enhanced phenotypic severity of the lung and lethality after birth. We also found that Podoplanin expression was detected in bronchial epithelium, mesothelium and a part of lymphatic endothelial cells during lung development.

Conclusion: CLEC-2 on platelets is essential for normal lung development.

Disclosure of Interest: None declared.

OR243

Non-genomic activities of retinoic acid receptor alpha control actin cytoskeletal events in human platelets

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Background: We previously demonstrated that anucleate human platelets are capable of performing nuclear functions, i.e. pre-mRNA splicing, progeny formation. Here, we demonstrate for the first time that human platelets (and megakaryocytes) constitutively express the retinoic acid receptor (RAR)- α , classically considered a nuclear transcription regulatory receptor.

Aims: Decipher the function of RAR α in human platelets.

Methods: RAR α expression, and interactions were analyzed using PCR and Co-IP techniques. Arp2/3 subunit 5 expression was studied using RNA deep sequencing approaches, immunocytochemistry, and duolink co-localization techniques. Actin branching was visualized using an *in vitro* actin branching assay utilizing recombinant proteins in conjunction with live microscopy experiments.

Results: We identify that in human platelets, RAR α interacts directly with Arp2/3 subunit 5, a component of the Arp2/3 complex. Arp2/3 subunit 5 is abundantly expressed in human platelets, co-localizes with RAR α , and regulates human platelet cytoskeletal functions. The high-affinity RAR α ligand all-trans-retinoic acid (atRA) disrupts RAR α -Arp2/3 interactions, inhibits platelet spreading, and blocks platelet progeny formation while preserving platelet activation responses. We further show that RAR α , when interacting with Arp2/3, acts as an inhibitory factor for actin nucleation and complex actin branch formation.

Conclusion: Our findings identify a previously unknown mechanism by which RAR α regulates Arp2/3-mediated actin cytoskeletal dynamics in human platelets through a non-genomic signaling pathway. These findings have broad implications in both nucleated and anucleate cells where actin cytoskeletal dynamics are integral to cell movement and division.

Disclosure of Interest: None declared.

OR244

Leukemia-associated rhogef (LARG) plays a minor role in rhoa activation, yet is critical for platelet activation in mice

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Background: Platelets express multiple members of the Rho-family of small GTPases whose role, as in other cell types, is to regulate actin cytoskeletal rearrangements and membrane and granule trafficking. RhoA is described as being an important Rho-family member in platelets, regulating platelet generation and function; however, much is still not known about the regulation of RhoA in platelets. LARG (AR-HGEF12) is a GPCR-regulated RhoGEF and is reported as being the most highly expressed RhoGEF in platelets. We hypothesized that LARG may therefore be an important regulator of RhoA activation in platelets.

Aims: To characterize the role of LARG in platelets.

Methods: A LARG conditional knockout driven by the PF4 promoter was used for analysis of platelet function and thrombosis.

Results: We have found that platelet-specific deletion of LARG results in an attenuation of platelet aggregation and secretion responses in response to the thromboxane receptor agonist, U46619, and the PAR4-receptor agonist, AYPGKF, but not ADP. Similarly, reduced thrombus formation was seen at low ferric chloride-injury levels *in vivo*. Interestingly, RhoA activation was largely unaffected by the absence of LARG. Haematological parameters and cell surface receptors were unchanged in the absence of LARG.

Conclusion: LARG is an important regulator of platelet function and thrombus formation *in vivo*, but does not appear to be a critical regulator of RhoA. This suggests that RhoA could be regulated by other RhoGEFs and/or LARG is also a GEF for other Rho-family members. The absence of any defect in haematological parameters or surface receptors suggests that, unlike RhoA, the absence of LARG does not affect platelet generation.

Disclosure of Interest: None declared.

OR245

Calpain-controlled detachment of major glycoproteins from cytoskeleton regulates adhesive properties of phosphatidylserine-positive activated platelets

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Background: In resting platelets, adhesive membrane glycoproteins are attached to the cytoskeleton. Upon strong activation, two platelet subpopulations are formed, phosphatidylserine(PS)-positive and PS-negative ones.

Aims: The objective of this study was to investigate the attachment of the key adhesive glycoproteins to platelet cytoskeleton in platelet subpopulation and the relationship between the attachment/detachment adhesive glycoproteins and adhesive properties of platelets.

Methods: We used a flow cytometry-based single-cell approach and Western blotting to investigate the attachment of membrane glycoproteins to the cytoskeleton in platelet subpopulations. Protein electrophoresis was used to examine the status of cytoskeletal proteins. Confocal microscopy was used for visualization of adherent platelets in a flow chamber.

Results: In PS-negative activated platelets, adhesive glycoproteins integrin α IIb β 3, glycoprotein Ib and, as shown for the first time, P-selectin were associated with cytoskeleton. In contrast, this attachment was disrupted in PS-positive activated platelets. It correlated with the degradation of talin and filamin observed only in PS-positive platelets as detected by protein electrophoresis of pre-sorted platelet subpopulations. Calpain inhibitors significantly decreased the disruption of the membrane glycoprotein attachment in PS-positive platelets, as well as talin and filamin degradation. Suggesting that the detachment of key glycoproteins from platelet cytoskeleton may affect adhesive properties of PS-positive platelets, we investigated the ability of PS-positive platelets to resist shear-induced detachment from the immobilized fibrinogen in a flow chamber. This resistance was very low, but it was increased more than 10-fold by pretreatment of platelets with calpain inhibitor MDL28170.

Conclusion: Our data suggest that calpain-controlled detachment of membrane glycoproteins negatively regulates adhesive properties of PS-positive activated platelets.

Disclosure of Interest: None declared.

Coagulation factors, V, X and XIII

OR246

Macrophage-specific receptor SR-AI plays a crucial protective role in the regulation of coagulation factor X plasma levels

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Background: Factor X (FX) is known to have a long circulating half-life (40 h) compared to structurally related hemostatic factors such as Factor VII (5 h). However, the mechanism regulating FX plasma levels remains poorly understood. Our laboratory demonstrated that FX binds specifically to Kupffer cells, macrophages present in the liver. Surprisingly, macrophage depletion in mice strongly reduced plasma levels of FX, indicating that macrophages play a protective role in the regulation of FX plasma levels.

Aims: To investigate the mechanism by which macrophages maintain FX plasma levels.

Methods: Duolink-PLA and classical immuno-staining were used to identify potential receptors for FX on macrophages (both primary and THP1-derived). FX-receptor interactions were further characterized using purified proteins, transfected cell lines and knock-out mice.

Results: Flow cytometry experiments using undifferentiated monocytes vs. macrophages demonstrated that FX selectively binds to macrophages but not their undifferentiated precursors. Duolink-PLA and immuno-confocal microscopy revealed that FX displays a high degree of co-localisation ($53 \pm 23\%$) with the Scavenger Receptor class AI (SR-AI), a macrophage-specific receptor. Binding experiments using purified proteins demonstrated a dose-dependent association of FX to immobilized SR-AI, which could be blocked by SR-AI specific inhibitors. Furthermore, FX bound specifically to SR-AI-expressing HEK293 cells but not to mock-transfected HEK293 cells. In addition, no significant binding of FX to macrophages obtained from SR-AI-deficient mice could be detected. Finally, endogenous FX plasma levels were decreased by $65 \pm 18\%$ in SR-AI-deficient mice compared to wild-type mice.

Conclusion: Altogether, our study demonstrates that FX is a ligand for macrophage-receptor SR-AI, and that binding to SR-AI is indispensable to maintain normal FX plasma levels.

Disclosure of Interest: V. Muczynski: None declared, A. Bazaa: None declared, A. Harel: None declared, C. Loubière: None declared, G. Chere: None declared, P. Lenting: None declared, C. Denis: None declared, O. Christophe Grant/Research Support from: Novo Nordisk A/S.

OR247

CB-FXa: an improved second generation FXa variant

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Background: Wild type (wt) human FXa, a key component of FEI-BA[®], acts 'downstream' in the coagulation cascade of Factors VIII and FIX. Consequently, FXa variants have the potential to become 'universal procoagulant agents' that could be used to treat both hemophilia inhibitor and normal patients (e.g., surgical bleeding or trauma).

Aims: To use structure-based rational design and molecular modeling to create a novel variant of human FXa (CB-FXa) that exhibits a significantly enhanced therapeutic index and improved efficacy and duration of action *in vivo*.

Methods: We measured the co-factor dependence of procoagulant activity (i.e., the ratio of catalytic efficiencies of a FXa protein for activation of prothrombin to thrombin in the presence or absence of FVa), the rate of inhibition by ATIII, and the efficacy for inhibition of bleeding ('tail clip' model) in FVIII^{-/-} mice. In addition, we performed single dose escalation toxicity/safety studies using wild type mice.

Results: CB-FXa maintained full procoagulant activity in the presence of co-factor (FVa) but displayed substantially decreased catalytic activity in the absence of co-factor. Consequently, the co-factor dependence of CB-FXa is enhanced by more than 450-fold compared with wt, human FXa and by more than 10-fold compared with FXa/I16L, a FXa drug candidate that is currently being tested in clinical trials. CB-FXa also exhibited more than 2000-fold resistance to inhibition by ATIII, compared with wild type FXa, and 40-fold greater resistance than FXa/I16L. In addition, data from murine efficacy and toxicity models demonstrated that CB-FXa displayed both significantly greater efficacy (14.5–25-fold) and therapeutic index (> 5-fold) *in vivo* than either wild type FXa or FXa/I16L.

Conclusion: Based on these improved *in vitro* and *in vivo* properties, CB-FXa appears to represent a novel second generation FXa variant that is significantly differentiated from both wt FXa and other advanced second generation FXa variants.

Disclosure of Interest: E. Madison Employee of: Catalyst Biosciences, Inc., C. Thanos Employee of: Catalyst Biosciences, Inc., M. Fox Employee of: Catalyst Biosciences, Inc., K. McCluskie Employee of: Catalyst Biosciences, Inc., J. Gureasko Employee of: Catalyst Biosciences, Inc., T. Byun Employee of: Catalyst Biosciences, Inc., D. Sloane Employee of: Catalyst Biosciences, Inc.

OR248

Characterization of a novel FV mutation (A512V, FV_{Bonn}) associated with deep vein thrombosis and APC resistance

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Background: Coagulation factor V (FV) has pro- and anticoagulant functions that make it a key regulator of haemostasis. Inactivation of FVa and FVIIIa by activated protein C (APC) is a major anticoagulant mechanism. FV_{Leiden} (R506Q) and other mutations associated with APC resistance are risk factors for venous thrombosis.

Aims: To characterise a novel F5 mutation (A512V, FV_{Bonn}), linked to APC resistance and clinically associated with deep vein thrombosis.

Methods: The FV_{Bonn} mutation was detected in 3 unrelated patients exhibiting APCR and deep vein thrombosis. The mutation was expressed transiently in a mammalian expression system. APC resistance was evaluated by aPTT and thrombin generation assay (TGT). The prothrombinase assay was performed to assess the APC inactivation rate of FV_{Bonn}. The cofactor activity of FV was analysed by a FVIII degradation assay.

Results: FV_{Bonn} conferred marked APC resistance to FV-deficient plasma, both in the aPTT (APCsr 1.98 for FV_{Bonn} vs. 1.59 for FV_{Leiden} and 4.31 for FV_{wt}) and TGT (nAPCsr 5.41 for FV_{Bonn} vs. 9.00 for FV_{Leiden} and 1.00 for FV_{wt}) assays. The APC-mediated inactivation of FVa_{Bonn} was partially impaired due to delayed cleavage at Arg⁵⁰⁶, and greatly stimulated by protein S. The APC-cofactor activity of FV_{Bonn} in FVIIIa inactivation was ~80% of that of FV_{wt}.

Conclusion: We have identified and characterized a novel F5 mutation associated with APC resistance and deep vein thrombosis. Although the FV_{Bonn} mutation does not directly affect an APC-cleavage site, it is located close to the Arg⁵⁰⁶ cleavage site and causes APC resistance with a mechanism similar to that of FV_{Leiden}. Decreased susceptibility to APC-catalysed inactivation and reduced APC-cofactor activity in APC-catalysed FVIIIa inactivation both contribute to FV_{Bonn} associated APC resistance.

Disclosure of Interest: None declared.

OR249

Platelet-derived factor V regulates arterial thrombosis

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Background: Coagulation factor V (FV) plays a key role in hemostasis, presents in plasma and platelets, and has a profound impact on thrombin formation. Patients with undetectable plasma FV seldom experience major bleeding, however, a causal role for FV in arterial thrombosis has not been established.

Aims: Our aim was to determine the role of platelet-derived FV in the regulation of arterial thrombosis.

Methods: Here, using transgenic mice with FV gene expression restricted to either the liver or megakaryocyte/platelet lineage. Mice were subjected to FeCl₃ carotid artery injury, and the time required to form an occlusive thrombus was measured.

Results: We found that time to arterial thrombotic occlusion was markedly prolonged in Tg+/-F5-/- mice (expressed approximately 15% of wild-type plasma FV level and undetectable platelet FV level). We observed that FV is expressed in platelets from Tg-/-F5 +/+ (expressed approximately 100% of wild-type plasma FV level and 100% of wild-type platelet FV level) and Tg-/-F5 +/- mice (expressed approximately 65% of wild-type plasma FV level and 50% of wild-type platelet FV level). Infusion of Tg-/-F5 +/+ mice platelets into Tg+/-F5-/- mice decreased the time to carotid artery occlusion after injury, indicating that platelet-derived FV directly regulates thrombosis.

Conclusion: Our data identify a molecular pathway of thrombosis that involves platelet FV and suggest that targeting FV has potential for treating atherothrombotic disorders.

Disclosure of Interest: None declared.

OR250

Development of a novel assay method of coagulation factor XIII activity for the detection of its inhibitor in plasma

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Background: Autoimmune hemorrhagic factor XIII (FXIII) deficiency (AH13) is a severe bleeding disorder due to anti-FXIII autoantibodies (autoAbs), and diagnosed cases of AH13 have recently been on the increase in Japan. We previously identified 40 AH13 cases and classified their anti-FXIII autoAbs into three types by their immunological properties. Type Aa and Ab autoAbs were anti-FXIII-A subunit (FXIII-A) IgGs and inhibited activation of FXIII and/or activity of activated FXIII, respectively. Inhibition of FXIII activity by anti-FXIII autoAbs was sometimes overlooked by either ammonia-release or amine-incorporation assay using small molecule substrates.

Aims: We developed a novel FXIII assay based on cross-linking of fibrin, to detect FXIII inhibitors much reliably.

Methods: Since incorporation of α_2 -plasmin inhibitor (α_2 PI) into fibrin clot was markedly reduced in AH13 cases, a fusion protein of the N-terminal portion of α_2 PI with alkaline phosphatase (PI-AP) was constructed as a reporter substrate. Plasma supplemented with PI-AP was reacted with thrombin and calcium, and AP activity incorporated into the clot was measured using chromogenic AP substrate.

Results: AP activity could be detected in fibrin clot generated from a complete reaction mixture of normal plasma but not that of FXIII-deficient plasma. FXIII-dose-dependent incorporation of PI-AP into the clot was confirmed by serial dilution of normal plasma with FXIII-deficient plasma. A five-step mixing test revealed an inhibition pattern of PI-AP incorporation in type Aa and Ab plasma where the inhibition was ambiguous in amine-incorporation and ammonia-release assays. This new assay allowed calculation of Bethesda units of inhibitors that correlated to other inhibitory profiles as well as reactivity to antigen.

Conclusion: New FXIII assay could detect the presence of FXIII inhibitors more reliably than traditional methods, and may be applied to a point-of-care-test of FXIII deficiency including AH13.

Disclosure of Interest: None declared.

Arterial vascular disease

OR251

The effect of intracoronary stem cell injection on markers of leukocyte activation in acute myocardial infarction

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Background: Beneficial effects of stem cell treatment during ST-elevation myocardial infarction (STEMI) have been suggested, but the effects on inflammation are controversial. The neutrophil cell markers pentraxin 3 (PTX3) and myeloperoxidase (MPO) are both reported to be elevated during STEMI.

Aims: To address the effects of stem cell treatment in STEMI on circulating and gene expression levels of PTX3 and MPO.

Methods: Subjects with STEMI undergoing percutaneous coronary intervention (PCI) were randomized to intracoronary injection of bone marrow cells (mBMC) ($n = 50$) or controls ($n = 50$). Blood samples were drawn one day before mBMC injection (baseline, median 6 days after PCI), after one day, three days, 2–3 weeks and 3 months. ELISA and RT-PCR were used for biochemical analyses. Myocardial necrosis was quantified by SPECT and levels of CKMB. The study was approved by the Regional Committee for Medical Research Ethics. All patients gave written informed consent to participate.

Results: PTX3 and MPO levels did not differ between the groups at any time point. Within the mBMC group, overall changes in both variables were observed ($P < 0.01$), with decreased levels from baseline throughout. Within the control group, similar patterns were observed. The relative reduction of PTX3 from baseline to Day 1 was significantly less pronounced in the mBMC group compared to controls ($P = 0.002$), whereas no differences in relative changes from baseline were observed for MPO. Plasma and gene expression levels of PTX3 in leukocytes correlated significantly at all time points ($r = 0.379$ – 0.448 , $P < 0.01$, all). Plasma levels of MPO correlated significantly to baseline LVEF ($r = -0.229$, $P = 0.025$) and peak CKMB ($r = 0.200$, $P = 0.05$).

Conclusion: Stem cell treatment had limited effect on plasma levels of PTX3 and MPO. The initially high PTX3 and MPO levels, the genetic regulation of PTX3 and the association between MPO and myocardial injury support the importance of neutrophil cell activation in STEMI.

Disclosure of Interest: None declared.

OR252

Platelet neutrophil complex formation and infiltration is regulated by peripheral serotonin in a mouse model of myocardial infarction

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Background: Reperfusion injury leads to platelet neutrophil complex (PNC) migration into cardiac tissue which affects the extent of necrosis after myocardial infarction (MI). Local serotonin levels are elevated during ischemia. Inflammatory response and neutrophil recruitment is attenuated in mice lacking peripheral serotonin (tryptophan hydroxylase-1-deficient mice, Tph1-/-).

Aims: Evaluate the effect of platelet serotonin on PNC formation and infiltration after myocardial ischemia reperfusion and its effect on necrosis and heart function.

Methods: WT and Tph1^{-/-} mice underwent surgery to induce MI. Reperfusion was allowed after 30 min. Heart function was determined by echocardiography after 24 h. Hearts were then excised to evaluate infarct size. PNC content was determined by flow cytometry and histology. Heart function of healthy WT and Tph1^{-/-} mice was analyzed in a working heart model. Cardiomyocytes were isolated from healthy WT and Tph1^{-/-} mice and survival was assessed over 48 h.

Results: Histology of frozen sections revealed a reduced number of PNCs in Tph1^{-/-} mice (16 ± 3) compared to WT (7 ± 3*). This was confirmed by flow cytometry of heart tissue (4 ± 2 in Tph1^{-/-}; 13 ± 1 in WT*) and blood (24 ± 8 in Tph1^{-/-}; 41 ± 15 in WT*). Infarct size was 37 ± 9% area at risk (AAR) in Tph1^{-/-} mice and 53 ± 13% AAR in WT mice (*). Echocardiography showed better heart function in Tph1^{-/-} compared to WT mice after surgery (49 ± 4%EF vs. 41 ± 6%EF*) whereas cardiac power after no flow in the ex vivo working heart assay was similar (33 ± 4 vs. 31 ± 3 mW gHW⁻¹). Isolated cardiomyocytes of Tph1^{-/-} and WT mice showed equal survival after 48 h (11.5% vs. 8.8%). *: $P < 0.05$.

Conclusion: Lack of platelet serotonin does not influence heart function of isolated hearts or survivability of cardiomyocytes *in vitro*. However, PNC formation and infiltration is reduced in Tph1^{-/-} mice resulting in attenuated reperfusion injury *in vivo*. Serotonin appears to account for up to 30% of myocardial reperfusion injury by promoting PNC formation.

Disclosure of Interest: None declared.

OR253

Post-PCI thrombin generation and cardiovascular mortality in acute coronary syndrome patients

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Background: Thrombin generation (TG) is a central step of the coagulation system involved in hemostatic and thrombotic roles, from promotion and inhibition of clotting, to fibrinolysis, platelet activation, and inflammation. No data are available on the possible role of thrombin generation on the prognosis of acute coronary syndrome (ACS) patients in the era of percutaneous coronary intervention (PCI) and stenting with dual antiplatelet therapy.

Aims: Aim of our study was to evaluate the possible association between the entity of thrombin generation and cardiovascular death in ACS patients undergoing PCI and stenting.

Methods: We investigated thrombin generation in 294 ACS patients (157 ST-elevation myocardial infarction [STEMI]; 137 Non-STEMI/Unstable Angina). TG was assessed using the calibrated automated thrombogram (CAT, Stago, France) and was expressed as endogenous thrombin potential (ETP), peak, and velocity index. Venous samples were obtained within 24 h from PCI.

Results: At one-year follow-up, 57 out of 294 patients (19.4%) died from cardiovascular causes. Higher values of ETP [1115.9 (705–1441.3) vs. 940.2 (666.0–1253.1) $P = 0.049$], peak [176.1 (80.5–259.4) vs. 107.3 (59.9–181.1) $P = 0.002$] and of velocity index [61.75 (21.03–97.88) vs. 25.64 (11.95–50.90) $P < 0.001$] were detected in patients dead during follow up compared to alive patients. At the multivariate model adjusted for the Global Registry of Acute Coronary Events (GRACE) risk score, the association between TG and cardiovascular death remained significant for peak [OR (95% CI): 2.341 (1.09–5.04) $P = 0.030$] and velocity index [OR (95% CI): 2.159 (1.01–4.63) $P = 0.048$].

Conclusion: In conclusion, high entity of thrombin generation, despite antithrombotic treatment and stent implantation, is an independent predictor of cardiovascular death and may be useful in improving risk stratification for ACS patients. Future prospective studies to evaluate the role of TG in determining cardiovascular events are warranted.

Disclosure of Interest: None declared.

OR254

Repeated measures of modifiable atherosclerotic risk factors over time and risk of myocardial infarction and venous thrombosis

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Background: Modifiable risk factors may influence the reported associations from cohort studies, especially when follow-up time is long.

Aims: To investigate whether changes in cardiovascular risk factors during follow-up affected the risk estimates for myocardial infarction (MI) and venous thromboembolism (VTE) in a population-based cohort with repeated measures.

Methods: The study included 6275 subjects enrolled in the fourth survey of Tromsø study (1994/95). Blood pressure, lipid levels, body mass index (BMI), diabetes and smoking status were measured at baseline, and subjects still alive at the fifth (2001/02, $n = 5376$) and sixth (2007/08, $n = 4391$) surveys were re-measured. Incident events of MI and VTE were recorded from enrollment to study end (December 2010). Conventional and time-varying Cox regression models were used to calculate hazard ratios (HR) for VTE and MI adjusted for age, sex and BMI. The study was approved by the research ethics committee and all subjects gave informed written consent.

Results: There were 848 incident MIs and 266 incident VTEs during follow-up. Variables that changed considerably over time were associated with the greatest changes in risk estimates when the time-varying model was compared to the conventional model. For MI, risk estimates associated with diabetes, smoking and physical activity changed from 2.55 (95% CI 1.96–3.32) to 1.91 (95% CI 1.50–2.43), from 1.80 (95% CI 1.57–2.08) to 2.07 (95% CI 1.79–2.39) and from 0.77 (95% CI 0.65–0.92) to 0.66 (95% CI 0.55–0.79), respectively. Variations in blood pressure and lipid levels were small over time, and did not alter the risk estimates in the time-varying model. BMI was the only factor associated with VTE, and the HR associated with obesity changed from 2.14 (95% CI 1.53–3.01) to 1.83 (95% CI 1.32–2.53) in the time-varying model.

Conclusion: For MI and VTE, risk estimates based on baseline and repeated measures corresponded well. Cardiovascular risk factors, except BMI, were not associated with VTE in time-varying analyses.

Disclosure of Interest: None declared.

OR255

Recurrence and mortality in young women with myocardial infarction or ischemic stroke: 19-year follow-up of the risk of arterial thrombosis in relation to oral contraceptives (RATIO) study

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Background: Data on long term follow-up of cardiovascular diseases in the young are scarce. Moreover, it is unknown if prothrombotic state affects the risk of recurrences.

Aims: To investigate the long term mortality and cardiovascular recurrences in young women who survived myocardial infarction (MI) or ischaemic stroke (IS).

Methods: Young women (< 50 years) with either MI ($n = 226$) or IS of noncardioembolic origin ($n = 160$) or no history of arterial thrombosis (i.e. healthy control subjects, $n = 782$) were followed between 1995 and 2012. Incidence rates (IR) and their ratios (IRR) for patients vs. controls were calculated for vascular events (MI or IS) and mortality. We used Cox regression to obtain hazard ratios (HR) adjusted for cardiovascular risk factors. A prothrombotic score summarising acquired and inherited procoagulant markers was used in a quartile analysis to determine the relationship between hypercoagulability and the risk of recurrence.

Results: During the median follow-up of 19 years, 83 deaths occurred. Mortality rates per 1000 person-years were 8.8 (95% Confidence Interval 6.2–12.3) in MI patients, 4.4 (95% CI 2.4–7.6) in IS patients, and 2.4 (95% CI 1.7–3.4) in controls. Cardiovascular events occurred in 44 MI patients (IR 12.1 per 1000 person-years; 95% CI 8.7–16.2), 37 IS patients (IR 14.1; 95% CI 9.9–19.4) and 13 controls (IR 0.9; 95% CI 0.5–1.5). Compared with controls, fully-adjusted HRs for cardiovascular events were 9.8 (95% CI 5.0–19.4) in MI patients and 12.9 (95% CI 6.7–25.0) in IS patients. Analysis of the score suggested that a prothrombotic state is associated with an increased risk of recurrence in IS patients (highest vs. lowest quartile adjusted HR 1.9, 95% CI 0.6–6.3), but not in MI patients (highest vs. lowest quartile HR 0.7, 95% CI 0.3–1.8).

Conclusion: Young women who survived MI or IS have a high long term mortality and morbidity. A prothrombotic tendency may increase the risk of cardiovascular recurrence after IS, but not after MI.

Disclosure of Interest: None declared.

Venous thromboembolism treatment – I

OR256

Residual vein thrombosis and the incidence of subsequent serious complications in patients treated with conventional anticoagulation

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Background: Deep-vein thrombosis (DVT) places a substantial number of patients at risk for long-term complications, such as recurrent venous thromboembolism (VTE), (severe) post-thrombotic syndrome (PTS), cancer and arterial thrombotic events. Whether residual vein thrombosis (RVT) can help identify patients at higher risk of these events is unknown.

Aims: To assess whether the ultrasound assessment of RVT can help predict serious adverse events occurring in patients with proximal DVT after the first three months.

Methods: We followed up for up to three years 869 consecutive patients with proximal DVT in a 7-year period. They received unfractionated or low-molecular-weight heparin followed by warfarin for 3–24 months. The assessment of RVT (defined as the incompressibility of at least 4 mm in the common femoral and/or the popliteal vein), was performed after three months. RVT was detected in 429 (49.4%) patients. Patients were censored after the uneventful completion of follow-up, at the time of death, loss to follow-up or development of events which we regarded as having comparable severity, i.e., recurrent VTE, severe PTS (skin ulcer or Villalta score > 14), overt cancer, acute myocardial infarction or ischemic stroke, whichever came first.

Results: Serious complications developed in 142 (33.1%) patients with and in 68 (15.5%) without RVT: recurrent VTE in 76 (17.7%) and 39 (8.9%); PTS in 26 (6.1%) and 15 (3.4%); cancer in 18 (4.2%) and 7 (1.6%); arterial disorders in 22 (5.1%) and 7 (1.6%), respectively. After adjusting for age, gender, extent of thrombosis, previous VTE, type of DVT (idiopathic or secondary) and duration of anticoagulation, the HR for serious complications in patients with as compared with those without RVT was 2.51 (95% CI, 1.87–3.36; $P < 0.001$).

Conclusion: In patients with proximal DVT, RVT increases the risk of serious long-term complications remarkably. Repeating ultrasonography after 3 months can help stratify the risk of patients and provide guidance for management decisions.

Disclosure of Interest: None declared.

OR257

Efficacy and safety of outpatient treatment based on the hestia clinical decision rule with or without nt-probnp testing in patients with acute pulmonary embolism: a randomized trial

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Background: Outpatient treatment of acute pulmonary embolism (PE) may lead to improved patient satisfaction and reduced health care costs. However, large scale trials to assess its safety and the optimal method for patient selection are scarce.

Aims: To investigate the safety of selecting PE patients for outpatient treatment by clinical criteria alone compared to clinical criteria combined with NT-proBNP testing.

Methods: Randomized non-inferiority trial conducted in 17 Dutch hospitals. Patients with proven acute PE were screened for outpatient treatment eligibility based on the Hestia criteria (Zondag et al., JTH 2011). Patients without any of the Hestia criteria were randomized to direct discharge or additional NT-proBNP testing. The latter patients were discharged as well if NT-proBNP was ≤ 500 ng L⁻¹ or admitted if NT-proBNP was > 500 ng L⁻¹. Primary endpoint was 30-day adverse outcome defined as PE or bleeding-related mortality, cardiopulmonary resuscitation or IC admission. Secondary endpoints were recurrent VTE, major bleeding and all-cause mortality.

Results: Between 2010 and 2013, 550 patients were randomized. In the NT-proBNP group, 34/275 (12%) had elevated NT-proBNP values and were managed as inpatients. The primary endpoint occurred in none of these 275 patients (0%; 95% CI 0–1.3%), vs. in 3/275 (1.1%; 95% CI 0.2–3.2%) of the patients in the direct discharge group ($P = 0.08$). These 3 patients all had normal NT-proBNP levels measured post-hoc. During 3-month follow-up, recurrent VTE occurred in 2 patients (0.73%; 95% CI 0.1–2.6%) in the NT-proBNP group vs. 3 patients (1.1%; 95% CI 0.2–3.2%) in the direct discharge group ($P = 0.65$). The rates of major bleeding were 0.4% vs. 1.1% ($P = 0.62$) and of all-cause mortality 1.5% vs. 1.1% ($P = 0.70$), respectively.

Conclusion: Outpatient treatment of PE patients selected by the Hestia criteria alone is safe. Additional prognostic assessment based on NT-proBNP levels would only change therapeutic management in 12% of patients, without affecting the 30-day prognosis.

Disclosure of Interest: None declared.

OR258

Comparison of ultrasound assisted catheter-directed thrombolysis (CDT) and CDT alone for treatment of proximal deep vein thrombosis

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Background: Recent studies suggested that catheter-directed thrombolysis (CDT) reduces development of postthrombotic syndrome (PTS). Experimental studies suggest that ultrasound-assisted CDT (USCDT) might enhance the efficiency of the thrombolytic process. However, the effect of USCDT on efficacy, PTS and quality of life (QOL) has not yet been studied well.

Aims: We compared USCDT with CDT on efficacy, safety, development of PTS and quality of life after long term follow-up.

Methods: We performed a retrospective cohort study of patients admitted with iliofemoral or inferior vena cava thrombosis to the University Hospital of Northern Norway from 2002 to 2011, who were treated with either CDT or USCDT. Participants alive were invited for a follow-up visit at the hospital from April 2013 until January 2014. Venography was performed to measure the degree of residual luminal obstruction of the affected veins. Each patient completed the generic Short-Form 36-item (SF-36) health survey assessment and the disease-specific Venous Insufficiency Epidemiological and Economic Study Quality of Life/Symptoms questionnaires. PTS was assessed using the modified Villalta scale.

Results: We included 94 patients. Mean age was 45 years (SD 17) and 31% were male. Baseline and risk factors for VTE were equally distributed between groups. In the USCDT group, we observed a significant decline in duration of thrombolytic treatment (< 48 h 27% vs. 6%), shortened hospital stay (mean 9.8 [SD 6.5] days vs. 7.1 [SD 2.9]), and less implantation of stents (30% vs. 55%). There was no difference in patency (76% vs. 79% patent) after follow-up (median 65 months range [15–141]). USCDT led to non-significant less severe-PTS (5% vs. 11%) and no differences in QOL. A non-significant incline in non-fatal major bleeding was observed (9% vs. 3%).

Conclusion: USCDT lead to shortened treatment, hospital stay, and less stenting. Trends of less severe-PTS and of increased major bleeding were observed with USCDT.

Disclosure of Interest: None declared.

OR259

Long-term anticoagulation with rivaroxaban for the prevention of recurrent deep venous thrombosis and pulmonary embolism: a benefit–risk analysis on the EINSTEIN EXTENSION trial

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Background: Clinicians balance the long-term risks of recurrent venous thromboembolism (VTE) once anticoagulation is stopped against the burden and risks of ongoing therapy.

Aims: To assess the benefit-risk tradeoff of continued rivaroxaban treatment in symptomatic VTE patients who completed 6–12 months of anticoagulation and in whom physicians had equipoise for needing continued anticoagulation.

Methods: In the EINSTEIN EXTENSION randomized, double-blind study, patients were assigned to rivaroxaban (20 mg once daily) or matching placebo for 6 or 12 months. Crude rates and risk differences (ITT population) of recurrent VTE and major bleeding were calculated using Mantel–Haenszel stratification and exact methodology, respectively. Benefits and risks were assessed using differences in rates in a hypothetical population of 10,000 patients and numbers needed to treat (NNT) and harm (NNH).

Results: In the rivaroxaban ($N = 602$) and placebo ($N = 594$) groups, recurrent VTE occurred in 8 and 42 patients (rates 1.3% and 7.1%). In a population of 10,000 patients, rivaroxaban treatment would have resulted in 574 (95% CI 349–800) fewer recurrent VTEs than placebo (NNT = 17). Major bleeding was observed in 4 (none fatal or in a critical organ) and 0 patients (rates 0.7% and 0). Rivaroxaban treatment would have resulted in 66 (95% CI 2–175) more major bleeds than with placebo (NNH = 151). Subgroup analyses showed a consistent pattern. Kaplan–Meier analysis showed that reduction in recurrent VTE with rivaroxaban started early and continued to improve throughout treatment, while major bleeding increased gradually and plateaued at ± 100 days.

Conclusion: In patients with symptomatic VTE who completed 6–12 months of anticoagulant therapy and in whom physicians did not deem continued anticoagulation necessary, a clinically important benefit and a favorable benefit–risk profile of continued anticoagulation with rivaroxaban was observed and would result in an important population benefit in recurrent VTE.

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OR260

A network meta-analysis comparing the efficacy and safety of anticoagulants for the treatment of venous thromboembolism in cancer patients

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Background: Current guidelines recommend low-molecular-weight heparin (LMWH) and vitamin-K-antagonists (VKA) for the treatment of venous thromboembolism (VTE) in cancer patients. Recently, non-vitamin-K-antagonist oral anticoagulants (NOAC) were approved as novel VTE treatment. However, their role in the cancer setting, particularly in comparison with the current standard of care, which is LMWH, remains unclear.

Aims: In this network meta-analysis we compared the relative efficacy and safety of LMWH, VKA, and NOACs for the treatment of VTE in patients with cancer.

Methods: We identified 10 randomized controlled trials including 3242 cancer patients. Recurrent VTE and major bleeding were defined as the primary efficacy and safety endpoint, respectively. Relative risks of these endpoints were pooled using a frequentist random-effects meta-regression model.

Results: In comparison to VKA, LMWH was associated with a significant reduction in the risk of recurrent VTE (RR = 0.60, 95% CI: 0.45–0.79, $P < 0.001$). Risks of major bleeding were comparable between LMWH and VKA (RR = 1.08, 95% CI: 0.70–1.66, $P = 0.74$). For the NOAC vs. VKA efficacy and safety comparison, the relative risk estimates were in favor of NOAC, but had confidence intervals that still included equivalence (RR for recurrent VTE = 0.65, 95% CI: 0.38–1.09, $P = 0.10$; RR for major bleeding = 0.72, 95% CI: 0.39–1.37, $P = 0.32$). In the indirect network comparison between NOAC and LMWH, the results indicated comparable efficacy (RR = 1.08, 95% CI: 0.59–1.95, $P = 0.81$), and a non-significant relative risk towards improved safety with NOAC (RR = 0.67, 95% CI: 0.31–1.46, $P = 0.31$). These results prevailed after adjusting for clinical heterogeneity between LMWH vs. VKA and NOAC vs. VKA studies.

Conclusion: This network meta-analysis suggests that the efficacy and safety of LMWH and NOACs for the long-term treatment of VTE in cancer patients may be comparable.

Disclosure of Interest: None declared.

ITP – Clinical

OR261

All-trans retinoic acid (ATRA) corrects the imbalance of macrophage polarization in patients with immune thrombocytopenia

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Background: Macrophages (M) can polarize into pro-inflammatory M1 or anti-inflammatory M2 phenotypes in response to different environment signals. Some autoimmune diseases are characterized by the impairment of macrophage polarization. However, there is no report referred to immune thrombocytopenia (ITP).

Aims: Our study aimed to investigate the imbalance of M1/M2 in ITP.

Methods: Human spleens and peripheral blood were obtained from ITP patients and healthy control. Splenocytes and peripheral blood mononuclear cells (PBMCs) were isolated. Human monocyte-derived macrophages were polarized to M1 or M2 *in vitro* by LPS plus IFN- γ or IL-4 with or without the addition of ATRA.

Results: An elevated M1/M2 ratio in splenocytes was observed in ITP patients compared with healthy control. Monocyte-derived macrophages induced from ITP patients showed higher expression of M1 surface markers (CD80, CCR7 and IL-12) and lower expression of M2 surface markers (IL-10, CD206 and CD36) than those from healthy control. For patients and control, the presence of ATRA potentiated the macrophage phenotype towards M2 by reducing CD80 and IL-12 expression and increasing CD206 and IL-10 expression. Macrophages obtained from ITP patients displayed a higher ability to promote both CD4⁺ T cell and CD8⁺ T cell proliferation and a lower ability to induce CD4⁺CD49b⁺LAG3⁺ regulatory T (Tr1) cells than those from healthy control, which could be corrected by ATRA. The concentration of IL-10 increased and IL-12 decreased in the co-culture supernatants of ATRA-modulated macrophages and T cells. Macrophages induced from ITP patients showed an elevated phagocytic capability, but no significant difference was observed between ATRA-modulated and unmodulated macrophages.

Conclusion: These findings demonstrated an imbalance of macrophage polarization towards M1 in patients with ITP, which could be corrected by ATRA. The study may shed lights on new mechanisms of ITP and provide basis for the therapeutic potential of ATRA in ITP patients.

Disclosure of Interest: None declared.

OR262

Retrospective analysis of rituximab therapy in childhood chronic and refractory immune thrombocytopenic purpura

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Background: Immune thrombocytopenic purpura (ITP) results from accelerated platelet destruction mediated by autoantibodies to platelet glycoproteins. Some patients with chronic ITP are refractory to all therapies (steroids, intravenous immunoglobulin (IVIG), anti-D and immunosuppressive drugs) and have chronic low platelet counts and episodic bleeding.

Aims: We retrospectively evaluated the efficacy and safety of rituximab treatment and splenectomy in pediatric patients diagnosed with

chronic and refractory ITP who were unresponsive to steroids, IVIG, cyclosporine, and mycophenolate mofetil.

Methods: Rituximab (Mabthera[®], Hoffmann-La Roche, Grenzach-Wyhlen, Germany) was administered in doses of 375 mg m⁻² week⁻¹ for four consecutive weeks, intravenously through a peripheral venous catheter in an inpatient setting. The responses were defined as follows: complete response, if the platelet count were > 100.000 mm⁻³; partial response, if the platelet count were 50.000–100.000 mm⁻³; minimal response, if the platelet count were lower than 50.000 mm⁻³ and higher than 30.000 mm⁻³, and no response if there were no increase in the platelet count. The responses to Rituximab therapy were evaluated in the 1st, 3rd, and 6th month after the treatment.

Results: Eight children and adolescents, with a median age of 12 years, with severe ITP were treated from January 2011 to December 2014. None of the 8 patients achieved a response. A splenectomy was performed in 6 of the 8 patients who had been treated with rituximab. Complete and partial responses were achieved in 67% and 33% of the patients, respectively. We evaluated the clinical characteristics and responses of chronic ITP patients who did not receive rituximab therapy and underwent a splenectomy. The success rate was 100% in the eight patients with chronic ITP.

Conclusion: Rituximab therapy might not be beneficial for some children with severe chronic ITP and AIHA who are refractory to standard agents. A splenectomy might be useful and preferable to rituximab.

Disclosure of Interest: None declared.

OR263

Occurrence of methemoglobinemia in immune thrombocytopenia patients treated with dapsone

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Background: Dapsone has been used in our center for the last 20 years as a second-line treatment for immune thrombocytopenia (ITP), with good results. There is limited experience with this agent in the medical literature, but dapsone is well tolerated and has low costs. The most recognized dapsone side effect is hemolysis, and it is also the first cause of drug-induced methemoglobinemia (MHb).

Aims: To evaluate the prevalence and clinical significance of MHb in ITP patients treated with dapsone.

Methods: MHb levels were measured by venous blood gas analysis. In 15 patients we measured MHb levels before the initiation of dapsone, and the maximum level found (1%) was used as the cut-off to define the MHb cases. Associations between MHb, dapsone dosage, hemolysis markers and platelet counts were analyzed by Spearman's correlation coefficient.

Results: Thirty-one patients were included in the study, with median age of 49 years. The study cohort consisted of: 87% primary and 13% secondary ITP; 90% chronic and 10% persistent ITP. Dapsone was prescribed as a fixed dose of 100 mg day⁻¹, dose alterations were made due to platelet responses or side effects. 97% of the patients developed MHb, but only 22% presented symptomatic MHb. The median MHb level found was 2.6% (0.8–18.2%). Among symptomatic cases, median MHb level was 6.8% (1.6–18.2%). None of the symptomatic patients required treatment, in 5 patients the drug was suspended and in one the dose was reduced with acceptable tolerance. MHb levels were associated with the drug dosage ($P = 0.002$; $r = 0.5$). We also found a significant negative correlation between platelet counts and MHb levels ($P = 0.007$; $r = -0.5$). There were no associations between MHb levels and hemolysis markers.

Conclusion: MHb is present in almost all ITP patients treated with dapsone and is usually asymptomatic. The consequences of chronic MHb in ITP patients treated with dapsone are unknown. The occurrence of

MHb has no association with the occurrence of hemolysis, but has a negative effect on platelet counts.

Disclosure of Interest: None declared.

OR264

Effect of CD72 on B cells in chronic primary immune thrombocytopenia

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Background: CD72, belonging to the C-type lectin family, is a coreceptor on B cells that can modulate the thresholds for signaling through the BCR for proliferation and differentiation. CD72 regulates BCR signaling both positively and negatively and exhibits some association with autoimmune diseases. However, the effect of CD72 on B cells in chronic primary ITP remains unclarified.

Aims: The aim of the study was to explore the effect of CD72 on B cells in chronic primary ITP.

Methods: Frequencies of B cell subsets were determined by flow cytometry in patients with chronic ITP before treatment ($n = 19$), after treatment ($n = 19$) and age-matched healthy individuals ($n = 20$). Expression of CD72 on B cells in ITP patients and controls was detected by flow cytometry. Peripheral blood mononuclear cells (PBMCs) were separated from patients and controls and labeled with CFSE. PBMCs were cultured in complete RPMI 1640 medium with or without anti-CD72 and meanwhile stimulated with IL-4, IL-10 and CD40 ligand. Proliferation of B cells was determined by flow cytometry. The cell culture supernatants were collected. Levels of secreted IgG were measured by ELISA.

Results: The frequency of naive B cell was significantly decreased in pre-treatment compared with post-treatment and controls ($P < 0.001$). However, there were significant increases in conventional memory B cell and pre-switched memory B cell in pre-treatment ($P = 0.018$, $P < 0.001$, respectively). Expression of CD72 on naive B cell increases both in chronic ITP patients and normal controls. The difference reached a statistical significance. Proliferation of B cells significantly increases in chronic ITP patients and can be inhibited by CD72. Level of IgG was decreased with anti-CD72 but the difference was not statistical.

Conclusion: Our findings suggest CD72 may be involved in class switch of B cell subsets. Moreover, CD72 has an inhibitive effect on proliferation and secreted antibody of B cells in chronic ITP. We conclude CD72 plays an important role in the B cells pathogenesis of chronic ITP.

Disclosure of Interest: None declared.

OR265

The effect of danazol and a new treatment strategy in primary immune thrombocytopenia – an analysis of 412 cases from a single center in China

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Background: Primary immune thrombocytopenia (ITP) is an acquired autoimmune disease. The current ITP treatment are limited by side effects, high relapse rate, or high costs. Danazol is an attenuated androgen and used as the second line treatment of ITP. There isn't a long-term and large-scale research about the effect of danazol in Chinese ITP patients till now.

Aims: This study aimed to assess the long-term benefit and side effects of danazol therapy, and determine the appropriate dose and duration of danazol.

Methods: This retrospective analysis includes 412 ITP patients in our center. 366 cases received danazol (50–300 mg) as initial therapy or added to the previous therapy. Glucocorticoids (GC) were tapered or discontinued. And 46 newly diagnosed ITP patients received danazol plus GC ($n = 25$) or GC alone ($n = 21$) in the early treatment. Clinical outcome and tolerance were assessed in all patients.

Results: Among the 366 cases of ITP, the overall response and complete response rate was 61.9% and 27.3% respectively. The median time to response was 1.8 ± 3 months. Age, phase of the disease, the time before danazol treatment, and the duration of danazol therapy influenced the response ($P < 0.05$). The median duration of remission was 18 ± 10 months, and 50.9% patients got sustained remission without danazol. The rate of relapse was 21.9%. A total of 20.1% patients experienced side effects, 4 cases discontinued due to side effects, others were well-tolerated with mild side effects. Among 46 newly diagnosed ITP patients, the response rate and response time between two groups were similar; but the rate of relapse was significant lower in the danazol plus GC group ($P < 0.05$).

Conclusion: Our study shows that low to medium dosage danazol is effective and better tolerated, but long-term therapy is necessary to maintain remission. Instead of being the second line treatment, low dosage danazol can be combined with GC in the initial stage, which can reduce the dosage and duration time of GC to achieve well-tolerated and long-term remission.

Disclosure of Interest: None declared.

Risk factors for venous thrombosis – II

OR266

Meta-analysis of 65,734 individuals identifies *TSPAN15* and *SLC44A2* as two new susceptibility loci for venous thromboembolism

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Background: Venous thromboembolism (VTE), including deep vein thrombosis and pulmonary embolism, is the third leading cause of cardiovascular disease deaths. Although genetic variants have been found associated with VTE, they explain only 5% of the heritability of the disease.

Aims: To identify additional VTE susceptibility genes by conducting a meta-analysis of genome-wide association studies (GWAS).

Methods: Twelve GWAS totaling 7507 VTE cases and 52,632 controls were included in the discovery phase in which 6,751,884 single nucleotide polymorphisms (SNPs) were tested for association with VTE. New SNP associations exceeding the genome-wide significance level of 5×10^{-8} were selected for replication in 3 independent case-control studies totaling 3009 VTE patients and 2586 controls.

Results: Nine loci reached the pre-specified genome-wide significance in the discovery scan. Six mapped to genes already known to be associated with VTE (*ABO*, *F2*, *F5*, *F11*, *FGG* and *PROCR*) whereas three had not been previously reported to be associated with VTE: *TSPAN15*, *SLC44A2* and *ZFPM2*. By contrast to *ZFPM2*, the associations observed at *TSPAN15* and *SLC44A2*, were robustly replicated. For *TSPAN15*, the lead risk allele was associated with an odds ratio (OR) of 1.31 for VTE ($P = 5.74 \times 10^{-11}$ in the discovery and $P = 2.21 \times 10^{-7}$ in the replication study). In *SLC44A2*, the lead risk allele was associated with an OR of 1.21 for VTE ($P = 5.59 \times 10^{-10}$ in the discovery and $P = 2.64 \times 10^{-7}$ in the replication cohort). In four cohorts totaling up to 15,249 individuals, there was no association between the two lead SNPs and 25 plasma biomarkers known to be associated with hemostasis and thrombosis.

Conclusion: We identified 2 loci (*TSPAN15* and *SLC44A2*) that had novel associations with VTE yet do not belong to canonical pathways leading to thrombosis and have not been associated with other cardiovascular endpoints. These findings may provide etiologic and mechanistic insights into VTE pathophysiology.

Disclosure of Interest: None declared.

OR267

Associations between atrial fibrillation and cause-specific risks of pulmonary embolism and stroke

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Background: Atrial fibrillation (AF) is a well-known risk factor for stroke. Recently, AF has also been identified as a risk factor for pulmonary embolism (PE), but the impact of AF on risks of stroke and PE in the same populations has previously not been evaluated.

Aims: To investigate the association between AF and future risks of PE and stroke in a population-based cohort.

Methods: Subjects ($n = 29\,975$) were recruited from three surveys of the Tromsø study and followed from enrollment (1994–1995, 2001–2002 and 2007–2008) to end of 2010. Incident events of AF, PE and stroke during follow-up were identified and validated. Information on potential confounders was obtained at baseline. Subjects were censored after the date of first event. Cox-regression models with age as time scale and atrial fibrillation as time-dependent variable were used to calculate cause-specific hazard ratios (HRs) for PE and stroke with 95% confidence intervals (CIs). The study was approved by the regional committees for research ethics and all subjects gave their informed written consent.

Results: There were 1356 subjects with AF, 234 with incident PE, and 1261 with incident stroke during a median of 15.5 years of follow-up. The risk of PE (HR 10.61; 95% CI, 5.84–19.28) and stroke (HR 4.98; 95% CI, 3.56–6.97) was substantially increased during the first 6 months after the AF diagnosis with crude incidence rates of 29.8 per 1000 person-years (PY) for PE and 19.1 for stroke, respectively. The risk remained elevated for both PE (HR 2.06; 95% CI, 1.21–3.35) and stroke (HR 2.40; 95% CI, 2.01–2.87) throughout the study period.

Conclusion: We found that AF is associated with increased risk of both stroke and PE, particularly during the first 6 months after the AF diagnosis. Both the absolute and relative risk estimates of PE were higher than for stroke. Our findings suggest that the risk of PE should be considered when assessing thromboembolic risk in AF patients.

Disclosure of Interest: None declared.

OR268

Glucocorticoid receptor gene and risk of venous thromboembolism

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Background: The glucocorticoid receptor (GR) gene haplotype 3, which entails the polymorphism GR-9β, is a common genetic variant with decreased cortisol sensitivity and is related to a more active proinflammatory system. This haplotype is associated with an increased risk of cardiovascular disease.

Aims: Since inflammation is also involved in the pathogenesis of venous thrombosis (VT) we questioned whether haplotypes of the GR gene with different cortisol sensitivities are associated with an increased risk of VT.

Methods: Using data from a large case-control study on VT (MEGA study, $n = 11,253$, consent and ethical approval obtained), polymorphisms in the GR gene which have previously been associated with altered glucocorticoid sensitivity (*BclI*, N363S, 23EK, and GR-9β) were determined and combined into 5 preexisting haplotypes (obtained in $n = 7,899$). Odds ratios (OR) with 95% confidence intervals (CI) for the risk of VT were calculated by logistic regression analysis.

Results: Persons heterozygous for the GR gene variant 23EK (404 cases, 370 controls) had a 1.3-fold increased risk of VT (OR 1.29; 95% CI 1.11–1.49), compared with patients without this variant. The OR for the risk of VT in persons homozygous for this variant (8 cases, 4 controls) was 2.36 (95% CI 0.71–7.84). Persons homozygous or heterozygous for haplotype 5, which entails the polymorphisms 23EK and GR-9β, had a 1.2-fold increased risk of VT (OR 1.21; 95% CI 1.01–1.45), compared with patients with no copies of haplotype 5. No associations were found for the other polymorphisms and haplotypes.

Conclusion: The polymorphism 23EK in the GR gene in haplotype 5 is associated with an increased risk of VT. Our findings suggest that genetically determined cortisol sensitivity might be involved in the pathogenesis of VTE, possibly due to its effect on inflammatory profile.

Disclosure of Interest: None declared.

OR269

F11 is associated with recurrent event of VTE in women: a prospective cohort study

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Background: The knowledge about genetic association on recurrence with venous thromboembolism (VTE) is sparse.

Aims: Our aim was to investigate if common genetic variants, previously shown to contribute to the prediction of first time thrombosis in women, were associated with risk of a recurrent event.

Methods: The Thromboembolism Hormone Study (TEHS) is a Swedish nationwide case-control study on first-time VTE conducted in 2002–2009 in women 18–64 years of age ($n = 2835$). TEHS was approved by the regional medical ethics committees and all participants signed an informed consent. A cohort of 1010 women was followed up until a recurrent event or 31/12/2011. The genetic variants *F5* rs6025, *F2* rs1799963, *ABO* rs514659, *FGG* rs2066865, *F11* rs2289252, *PROC* rs1799810 and *KNG1* rs710446 together with clinical variables and family history of VTE were assessed. Incidence rate of recurrent event was calculated as the number of events over the accumulated patient-time. Cumulative incidence was calculated by Kaplan–Meier survival analysis. Cox proportional-hazard model was used to estimate hazard ratios (HR) and 95 percent confidence intervals (95% CI) between groups of women.

Results: One hundred and one recurrent events occurred during a mean follow-up time of 5 years accumulating a volume of 5094 person-years. The overall incidence of recurrence was 20 per 1000 person-years (95% CI; 16–24). The incidence was highest in women with unprovoked first event and obesity with a rate of 33 and 30 per 1000 person-years. Carriers of *F5* rs6025 and *F11* rs2289252 had significantly higher rates with HR = 1.7 (95% CI; 1.1–2.6) and 1.8 (95% CI; 1.1–3.0), respectively, independently of family history of VTE. The cumulative risk of recurrence was 2.5-fold larger in carriers of both *F5* rs6025 and *F11* rs2289252 than in non-carriers at 2 and 5 years into the study.

Conclusion: To our knowledge, this is the first time *F11* rs2289252 was found to be associated with risk of recurrent event with VTE in women.

Disclosure of Interest: None declared.

OR270

Role of apolipoprotein(a) kringle-IV type 2 copy number variation in venous thromboembolism

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Background: An association between high lipoprotein(a) [Lp(a)] concentrations and venous thromboembolism (VTE) is being evidenced. Lp(a) is controlled by genetic variants in *LPA* gene, coding for the apolipoprotein(a) moiety of Lp(a).

Aims: Aim of this study was to investigate the role of *LPA* gene kringle-IV type 2 (KIV2) size polymorphism and single nucleotide polymorphisms (SNPs) (rs1853021, rs1800769, rs3798220, rs10455872) in modulating the susceptibility to venous thromboembolism.

Methods: Five hundred and sixteen patients with idiopathic VTE without thrombophilic risk factors [median age 44 (9–80) years, 39% males] and 1117 healthy control subjects, comparable for age and gender, were investigated. *LPA* KIV2 polymorphism, rs3798220 and rs10455872 SNPs were genotyped by real time PCR TaqMan technology. Concerning rs1853021 and rs1800769 SNPs, PCR-RFLP assay was used.

Results: *LPA* KIV2 repeat number has been found to be significantly lower in patients than in controls [11 (0–116) vs. 15 (1–118), $P < 0.0001$]. In the VTE patients group, a significantly higher percentage of subjects with KIV2 repeat number ≤ 9 was observed with respect to controls (38% vs. 18%, $P < 0.0001$). KIV2 repeat number was independently associated with VTE ($P = 3.06 \times 10^{-11}$), as evidenced by general linear model analysis adjusted for traditional risk factors. No significant difference in allele frequency for all SNPs inves-

tigated was evidenced. Haplotype analysis showed that *LPA* haplotypes rather than individual SNPs influenced disease susceptibility.

Conclusion: In conclusion, our data indicate that *LPA* genetic variants, known to contribute to Lp(a) concentrations, are significant and independent susceptibility factors to VTE.

Disclosure of Interest: None declared.

Platelets – II

OR271

The direct dengue virus-platelet interaction: generation of infectious progeny

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Background: Dengue virus (DENV), a positive stranded RNA virus, causes ~200 million cases of severe flu-like illness annually, with ~500,000 escalating to life-threatening hemorrhagic fever or shock syndrome. The molecular basis of DENV disease involves many links to hemostasis, including thrombocytopenia.

Aims: To help understand DENV infection and contributions to pathology, we hypothesized that translation-competent platelets may serve as a replication center for DENV. The platelet-DENV interaction, platelet-dependent amplification of the DENV genome, and generation of infectious DENV associated with platelets were evaluated.

Methods: DENV serotype 2 was propagated in Vero cells and purified by differential ultracentrifugation. Leukocyte-free washed platelets were quantified and characterized for activation by flow cytometry. DENV2-platelet binding, genome replication and viral antigen production were quantified by qRT-PCR and immunoblots. Viable DENV2 progeny generation was determined using standard plaque assays.

Results: After correction for internalization and non-specific binding, DENV2 was found to have ~800 specific surface sites per platelet at 37 °C and was temperature dependent. Binding was partially inhibited by anti-DC-SIGN or low molecular weight heparin (~80%) alone, while their combination caused complete inhibition. Non-immune IgG, Fc and chondroitin sulfate had no effect. Platelets replicated the genome of DENV2 by up to ~5-fold after 7 days and produced virus-encoded NS1 protein. Infectious DENV2 was propagated by platelets, which was inhibited using the translation elongation inhibitor, cycloheximide.

Conclusion: For the first time, these data show saturable DENV-platelet association leading to viral genome replication, translation and production of viable progeny, adding to the pathogen-induced mechanism contributing to thrombocytopenia.

Disclosure of Interest: None declared.

OR272

The PH-domain adaptor Bam32/DAPP1 restrains GPVI-mediated platelet activation and thrombus formation

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Background: Phosphoinositide 3-kinases (PI3Ks) are required for physiological platelet adhesion and activation, acting downstream of several platelet receptors to generate 3-phosphoinositide messengers. Although the underlying mechanism by which PI3Ks regulate platelet

function is still largely unknown, it likely involves recruitment of PH domain-containing proteins to the membrane. We recently identified B cell adaptor molecule of 32 kDa (Bam32/DAPP1) as a major PH domain-containing PI(3,4)P₂- and PI(3,4,5)P₃-binding protein in human platelets.

Aims: To determine the contribution of the PH domain adaptor Bam32 to thrombosis and its regulation in platelets.

Methods: Human and murine platelet Bam32 expression and phosphorylation were assessed by immunoprecipitation and Western blotting. Platelet function was examined using flow cytometry, aggregometry and *in vitro* thrombus formation using platelets from wild-type and Bam32^{-/-} mice.

Results: Human and murine platelets express Bam32. The platelet agonists thrombin and collagen-related peptide (CRP) stimulated rapid serine phosphorylation in a PKC-dependent manner. Tyrosine phosphorylation of Bam32, which is responsible for Bam32-mediated effects in other blood cells, was slower and occurred downstream of PI3 kinase and Src kinases. Platelets from Bam32^{-/-} mice showed a marked increase of surface expression of both P-selectin and activated integrin $\alpha_{IIb}\beta_3$ in response to CRP, but not to a PAR4 thrombin receptor peptide. Furthermore, CRP-mediated aggregation of Bam32^{-/-} platelets was significantly enhanced compared to wild type. Finally, whole blood from Bam32^{-/-} mice showed significantly enhanced thrombus formation *in vitro* when flowed over a collagen-coated surface.

Conclusion: Bam32 is a negative regulator of GPVI-mediated platelet function and thrombosis. Our results suggest that PI3 kinase-mediated tyrosine phosphorylation of Bam32 attenuates GPVI signalling, thereby limiting the extent of thrombus formation.

Disclosure of Interest: None declared.

OR273

Impaired platelet activation but intact hemostasis in mice expressing low levels of the Rap-GEF, CALDAG-GEFI

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Background: The tight regulation of platelet adhesiveness, mediated by the $\alpha_{IIb}\beta_3$ integrin, is critical for hemostasis and the prevention of thrombosis. We recently demonstrated that integrin affinity in platelets is controlled by the guanine nucleotide exchange factor, CalDAG-GEFI (CD-GEFI), and its target Rap1. Mice deficient in CD-GEFI were protected from thrombosis but also exhibited a significantly impaired hemostatic response.

Aims: In this study, we investigated whether low-level expression of CD-GEFI leads to protection from thrombosis without pathological bleeding in mice.

Methods: CD-GEFI^{low} mice were generated by knock-in of human CD-GEFI cDNA into the mouse locus. Integrin activation response to various platelet agonists was assessed by flow cytometry and standard aggregometry. Hemostasis in mice was assessed by standard tail-clip assay and a novel saphenous vein laser injury model recently described by our group. Thrombosis was assessed in the ferric chloride carotid artery injury model.

Results: CD-GEFI expression in platelets from CD-GEFI^{low} mice was reduced by ~90% when compared to controls. Integrin activation in CD-GEFI^{low} platelets, measured by JON/A-PE binding, was abolished at low agonist concentrations and partially inhibited at high agonist concentrations. Consistent with this observation, the aggregation response of CD-GEFI^{low} platelets was weaker than that of WT platelets, but more efficient than that observed in CD-GEFI^{-/-} cells.

Importantly, CD-GEFI^{low} mice were protected from arterial thrombosis, with only minimal impact on primary hemostasis.

Conclusion: Together, our studies suggest partial inhibition of CD-GEFI function as a powerful new approach to safely prevent thrombotic complications.

Disclosure of Interest: None declared.

OR274

The calcium-binding protein S100A1 negatively regulates collagen-dependent platelet activation and thrombosis in mice

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Background: S100A1 is a member of the S100 family of calcium-binding proteins. S100A1 controls Ca²⁺ dynamics in cardiomyocytes and plays an important role in heart failure. S100A1 is also expressed in mouse platelets, but its role in platelet biology has not been investigated.

Aims: To determine the role of S100A1 in platelet activation and thrombosis.

Methods: Platelet activation was measured by flow cytometry and aggregation in whole blood or washed platelets from wild-type (WT) or S100A1-deficient (SKO) mice. Thrombus formation was evaluated by flow chamber studies and by the *in vivo* FeCl₃ carotid artery thrombosis model.

Results: Platelet activation in response to threshold levels of convulxin, a specific agonist for the collagen receptor GPVI, showed significantly increased activation of α IIB β 3 integrin and α -granule release in SKO platelets compared with WT platelets. Consistently, SKO platelets also showed a more robust aggregation response to convulxin and collagen. In contrast, SKO platelets responded normally to stimulation with PAR4 receptor-activating peptide or ADP. Adhesion of SKO platelets to collagen under flow conditions was not significantly different to that of WT platelets. However, we observed a ~3-fold increase in phosphatidylserine positive SKO platelets bound to the collagen surface. We also observed increased coated platelet formation and more sustained calcium transients in SKO platelets compared to controls. The increased reactivity of SKO platelets to GPVI agonists is explained by a ~1.5-fold increase in GPVI receptors expressed on the surface of these cells. A similar increase in GPVI expression was also found in bone marrow-derived megakaryocytes. When subjected to the FeCl₃ carotid artery thrombosis model, the time to vessel occlusion was significantly shorter in SKO mice compared to WT controls.

Conclusion: We here identify S100A1 as a negative regulator of GPVI expression and collagen-dependent platelet activation and thrombosis in mice.

Disclosure of Interest: None declared.

OR275

Dissecting roles for the SNARE-associated protein, SNAP29, in mouse platelets

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Background: Secretion of platelet granule cargo is critical for efficient platelet function. Understanding the regulation of the processes underlying secretion is therefore essential for our understanding of thrombotic and hemostatic disease processes and for the development of effective therapeutics. It has been previously demonstrated that SNARE complex members such as SNAP23, VAMP8 and syntaxin 11, etc., play significant roles in platelet secretion. Of other SNARE complex members expressed in platelets, a role for the tSNARE SNAP29 has yet to be described, despite being the second most highly expressed SNAP-family member in platelets. We hypothesized that SNAP29 may play an important role in regulating granule secretion in platelets.

Aims: To characterize the role of SNAP29 in platelets.

Methods: A SNAP29 conditional knockout driven by the PF4 promoter was used for analysis of platelet function and thrombosis.

Results: We have found that platelet-specific deletion of SNAP29 does not result in an attenuation of platelet aggregation or secretion responses in response to thrombin or collagen-related peptide (CRP). Intracellular signalling was also not perturbed in the absence of SNAP29. Primary thrombus formation was unaffected *in vivo* and *in vitro*, however there was an increased number of emboli observed *in vivo*, suggesting increased thrombus fragility. Static adhesion to CRP was also diminished in the absence of SNAP29.

Conclusion: SNAP29 does not appear to be a critical regulator of platelet secretion, however, it does regulate thrombus stability *in vivo*. This could be due to regulation of adhesion to collagen, signifying that SNAP29 could modulate collagen-dependent signalling in a context-specific manner in certain pathophysiological settings.

Disclosure of Interest: None declared.

Platelets – kinases – II

OR276

A comparison of Btk/Tec kinases in platelets using ibrutinib and knockout mice

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Background: Ibrutinib is an irreversible inhibitor of the Btk/Tec family protein tyrosine kinase Btk, which is currently in use in the clinic for the treatment of several B-cell malignancies. Btk has previously been shown to play a redundant role with Tec downstream of the collagen receptor, GPVI, and a non-redundant role downstream of the VWF receptor, GPIb.

Aims: We have used ibrutinib, and knockout platelets, to investigate the role of these kinases downstream of the podoplanin receptor, CLEC-2. We subsequently used ibrutinib to identify Btk substrates.

Methods: Btk/Tec-deficient mice were analysed for developmental and platelet function defects using standard assays. Signalling in platelets

treated with ibrutinib was studied by Western blotting using several phospho-specific antibodies.

Results: Btk/Tec-deficient mice show characteristic blood-lymphatic mixing in the intestinal vasculature, suggestive of a role downstream of CLEC-2. The platelets from these mice show defective aggregation, P-selectin exposure, and spreading in response to CLEC-2 ligands. Pre-treatment of platelets with ibrutinib inhibited platelet aggregation to both CLEC-2 and GPVI agonists, in association with loss of phosphorylation of PLC γ 2 at Y1217. Ibrutinib inhibited the Btk auto-phosphorylation site (Y223) confirming activity and specificity of the drug. Paradoxically, we found that ibrutinib treatment resulted in hyper-phosphorylation of several proteins, namely Syk (Y352 + Y525/6), LAT (Y171), SLP-76 (Y145), and interestingly, Btk itself on the Src phosphorylation site (Y551).

Conclusion: These results demonstrate that Btk/Tec kinases play a role in platelets downstream of CLEC-2, as they do for GPVI, and are involved in the development of the lymphatic system. They also show that targeting Btk with ibrutinib results in platelet inhibition, most likely due to a decrease in the activation of the Btk substrate PLC γ 2. The associated hyper-phosphorylation of several other signalling proteins is unexpected and will be further investigated.

Disclosure of Interest: None declared.

OR277

PP1c alpha negatively regulates platelet thrombus formation via MAPK p38

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Background: Signal transduction mediated by kinases and phosphatases are critical for platelet activation. The contribution of serine/threonine phosphatases to platelet function have lagged behind kinases. Our understanding of how the catalytic subunit of protein phosphatase 1 (PP1c) contributes to platelet biology has largely been confined to studies with pharmacological agents. While a useful tool, inhibitors often lack specificity to discriminate PP1c isoforms. PP1c α is a major platelet expressed isoform and its role in platelets remains unexplored.

Aims: To clarify the contribution of PP1c α in platelet functions using a genetic approach.

Methods: By crossing the PP1c α ^{flax/flax} mice with PF4^{Cre} mice, we generated the platelet specific PP1c α ^{-/-} mice and analyzed platelet function.

Results: Characterization studies confirmed that platelets from these mice only lack the PP1c α but retained PP1c β and PP1c γ . PP1c α ^{-/-} platelets displayed decreased aggregation to low doses of agonists, including thrombin, ADP, collagen, convulxin and U46619. These studies suggest that PP1c α positively regulates integrin α _{IIb} β ₃ inside-out signaling. Biochemical studies revealed that PP1c α co-immunoprecipitated with p38 mitogen activated protein kinase (MAPK). PP1c α ^{-/-} platelets showed enhanced fibrin clot retraction and adhesion to immobilized fibrinogen under static conditions. Interestingly, whole blood from PP1c α ^{-/-} mice perfused over collagen at a shear rate of 1000 s⁻¹ revealed increased thrombus formation. Importantly, p38 inhibitor blocked the increased functional phenotypes (clot retraction and thrombus formation) of PP1c α ^{-/-} platelets. These studies suggest that loss of PP1c α in platelets upregulates p38 MAPK, which contributes to the enhanced outside-in α _{IIb} β ₃ signaling dependent functions.

Conclusion: Unlike the generic Ser/Thr phosphatase inhibitors block all platelet functions, we show that PP1c α positively regulates inside-out signaling and negatively regulates outside-in α _{IIb} β ₃ signaling via p38 MAPK.

Disclosure of Interest: None declared.

OR278

DUSP3 phosphatase deficiency or inhibition limit platelet activation and arterial thrombosis

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Background: A limitation of current antiplatelet therapies is their inability to separate thrombotic events from bleeding occurrences. Recently, protein tyrosine phosphatases (PTPs) have emerged as critical regulators of platelet function.

Aims: 1/To Investigate the function of DUSP3 in thrombosis and hemostasis. 2/To develop DUSP3 specific inhibitor towards the generation of novel antiplatelet agents.

Methods: Platelet functions were performed using DUSP3-KO and wild mice-type platelets. Aggregate formation on collagen surface was analyzed under flow conditions. Arterial thrombosis was assessed in a model of pulmonary embolism and upon ferric chloride induced carotid artery injury. To identify DUSP3 inhibitors, high-throughput screening (HTS) was employed using a colorimetric phosphatase assay with *p*-nitrophenolphosphate (pNPP) as substrate, and screened 291,018 drug-like molecules.

Results: We show that the dual-specificity phosphatase 3 (DUSP3) is highly expressed in human and mouse platelets. Platelets from DUSP3-deficient mice displayed a selective impairment of aggregation and granule secretion mediated through GPVI and CLEC-2 receptors. DUSP3-deficient mice were more resistant to collagen- and epinephrine-induced thromboembolism, compared to wild-type mice, and showed severely impaired thrombus formation upon ferric chloride-induced carotid artery injury. DUSP3 deficiency impaired Syk tyrosine phosphorylation, subsequently reducing phosphorylation of PLC γ 2 and calcium fluxes. To investigate DUSP3 function in human platelets, a novel small-molecule inhibitor of DUSP3 was developed. This compound specifically inhibited collagen and CLEC-2-induced human platelet aggregation, thereby phenocopying the effect of DUSP3 deficiency in murine cells.

Conclusion: DUSP3 plays a selective and essential role in collagen- and CLEC-2-mediated platelet activation and thrombus formation *in vivo*. Inhibition of DUSP3 may prove therapeutic for arterial thrombosis.

Disclosure of Interest: None declared.

OR279

Class III PI3K positively regulates platelet activation and thrombus formation via PtdIns3P directed function of the NADPH oxidase

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Background: Class III PI3K, also known as Vps34, is a highly conserved enzyme in eukaryocytes. By synthesizing the messenger lipid product PtdIns(3)P, Vps34 is involved in biological functions such as membrane trafficking and autophagy. We have previously shown that platelet is equipped with a set of autophagic proteins including Vps34.

Aims: To investigate the function and mechanism of Vps34 in platelet activation and thrombosis.

Methods: By platelet specific Vps34 deficiency mice and using a Vps34 antagonist 3-methyladenine in human platelets, phenotype and signaling alterations of Vps34 deficiency were characterized.

Results: Platelet-specific Vps34 deficient mice exhibited a prolonged vessel occlusion times upon ferric chloride-induced mesenteric arteriole injury, while the tail bleeding time was not altered. Thrombus formation on a collagen matrix under arterial shear conditions was significantly reduced for Vps34^{-/-} platelets. Vps34-deficient platelets also displayed an impaired aggregation and dense granule secretion in response to collagen and thrombin stimulation. Spreading on immobilized Fg and the rate of clot retraction in platelet-rich plasma were both reduced for Vps34^{-/-} platelets. Characterization of signaling events revealed that Vps34 deficiency reduced PtdIns(3)P production upon platelet activation, which subsequently hampered the phosphorylation (Thr154) of a key component of NADPH oxidase p40phox and decreased reactive oxygen species generation. Furthermore, Vps34 deficiency leads to a decreased phosphorylation levels of Akt (Ser473), but not Akt (Thr 308) upon collagen and thrombin stimulation. The phenotype and signaling defects of Vps34 deficiency in murine platelets were confirmed by using a Vps34 antagonist 3-methyladenine in human platelets.

Conclusion: The data presented here reveal that Vps34-dependent NADPH oxidase activity is an important regulator for platelet activation and thrombus formation *in vivo*. Targeting Vps34 may be a new strategy for the prevention of arterial thrombosis.

Disclosure of Interest: None declared.

OR280

AADACL1 regulation of PKC-dependent secretion in human platelets

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Background: We previously discovered a novel lipid hydrolase named arylacetamide deacetylase-like 1 (AADACL1) in human platelets that regulates signaling downstream of multiple agonists, especially collagen. Our work and that of others suggests that lipid substrates of AADACL1 such as 2-acetyl monoalkylglycerol ether (2-acetyl MAGE) and its deacetylated product, MAGE, modulate PKC function.

Aims: We hypothesize that AADACL1 regulates platelet secretion and activation via direct interactions between 2-acetyl MAGE and/or MAGE with lipid-binding C1 domains of platelet PKC isoforms.

Methods: Lipid monomers or small unilamellar vesicles (SUV) were assayed for binding to PKC peptides using intrinsic tryptophan fluorescence. Lipid modulation of purified PKC kinase activity was also tested and PKC activation status in platelets was measured with phosphorylation site-specific antibodies. Platelet secretion was determined by lumi-aggregometry.

Results: Both 2-acetyl MAGE and MAGE SUV bound the PKC δ C1b domain with high affinity, similar to diacylglycerol, a known PKC activator. Although MAGE displayed tighter binding to PKC δ compared to 2-acetyl MAGE, MAGE did not affect PKC δ kinase activity, whereas 2-acetyl MAGE inhibited kinase activity *in vitro*. Consistent with this, exogenous 2-acetyl MAGE and an AADACL1-selective inhibitor, JW480, each reduced dense granule secretion in stimulated platelets, but MAGE did not. Addition of exogenous ADP bypassed JW480-mediated aggregation defects, strongly suggesting that AADACL1 regulates ADP release from platelet granules. Inhibition of AADACL1 also correlated with decreased PKC δ but not PKC θ phosphorylation in platelets.

Conclusion: AADACL1 deacetylates an inhibitory lipid that may compete with endogenous lipid activators of PKC. Thus, AADACL1 promotes efficient and irreversible platelet activation at low agonist concentrations by removing a previously unrecognized constraint on

PKC activity and subsequent dense granule secretion of agonists such as ADP in human platelets.

Disclosure of Interest: None declared.

Non-vitamin K oral anticoagulants and surgery

OR281

Impact of oral anticoagulation on time to procedure in patients admitted for hip fracture

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Background: Hip fracture is associated with significant morbidity and mortality particularly if surgical repair is delayed. Current guidelines recommend performing surgery within 48 h of admission. Patients with hip fractures frequently receive oral anticoagulation (vitamin K antagonists (VKA) or direct oral anticoagulants (DOACs), which may complicate perioperative management. Reversal strategies for VKA are well-established (vitamin K and/or prothrombin complexes (PCC)) while they are controversial for DOACs.

Aims: To determine whether the time to surgery for hip fracture is different in patients receiving anticoagulation (VKA and/or DOACs).

Methods: Case-control study of consecutive patients on oral anticoagulation (VKA or DOACs) admitted to hospital for surgical repair of hip fracture ($n = 260$) between January 2005 and March 2014. Controls ($n = 260$) were matched for age and gender. The primary outcome was the time from admission in the emergency room to surgery. Secondary outcome measures included: 1) venous thromboembolism (VTE); 2) major bleeding; and 3) in-hospital overall mortality.

Results: Of 260 patients on oral anticoagulation included in the study, 233 (90%) were on VKA whereas the others were on a DOAC. Patients receiving any form of anticoagulation experienced a longer median delay in hip surgery. (40.1 vs. 26.2 h ($P < 0.001$)) compared to controls. Similarly, patients on DOACs experienced a longer median wait time as compared to patients on VKA or no anticoagulation. (66.9, 39.4 and 26.2 h respectively ($P < 0.001$)). There were no differences in the rates of VTE, major bleeding episodes or in-hospital overall mortality between the different groups.

Conclusion: Despite the availability of reversal methods (vitamin K or PCC), patients receiving anticoagulation seem to experience significant delays in surgical repair of their hip fracture. Patients on DOACs seem to experience longer delays compared to patients on VKAs. However, no increases of adverse events were observed in the post-operative period.

Disclosure of Interest: None declared.

OR282

Perioperative management of dabigatran: a prospective cohort study

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Background: The perioperative management of dabigatran in clinical practice is heterogeneous.

Aims: We performed this study to evaluate the safety of perioperative management of dabigatran using a specified protocol.

Methods: Patients treated with dabigatran and planned for an invasive procedure were eligible for inclusion. The timing of the last dose of dabigatran before the procedure was based on the creatinine clearance and procedure-related bleeding risk. Resumption of dabigatran was prespecified according to the complexity of the surgery and consequences of a bleeding complication. Patients were followed for 30 days for major bleeding (primary outcome), minor bleeding, arterial thromboembolism and death.

Results: We included 542 cases; 324 procedures (60%) with standard risk of bleeding and 218 procedures (40%) with increased risk of bleeding. The last dose of dabigatran was at 24, 48, or 96 h before surgery according to the protocol in 46%, 37% and 6%, respectively, of the cases. Dabigatran was resumed a median of 1 day (interquartile range, 0–2) after the procedure. Resumption was timed according to protocol in 77% with 75 mg as the first dose on the day of procedure in 40% of the cases. Ten patients (1.8%; 95% confidence interval [CI], 0.7–3.0) had major bleeding and 28 patients (5.2%; 95% CI, 3.3–7.0) had minor bleeding events. The only thromboembolic complication was transient ischemic attack in 1 case (0.2%; 95% CI, 0–0.5), and there were 4 deaths unrelated to bleeding or thrombosis. Bridging was not used preoperatively but was administered in 9 cases (1.7%) postoperatively.

Conclusion: Our protocol for perioperative management of dabigatran appears safe, effective and feasible.

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OR283

An observational cohort study to evaluate the safety and efficacy of dabigatran etexilate in patients with moderate renal impairment undergoing elective total hip or knee replacement surgery

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Background: In clinical trials, dabigatran etexilate (DE) 220 or 150 mg once daily (qd) was as effective as enoxaparin for prevention of venous thromboembolism (VTE) in patients undergoing elective total hip/knee replacement (THR/TKR) with a similar safety profile. The usual recommended dose is 220 mg qd. A lower dose (150 mg qd) is recommended for patients with moderate renal impairment (RI), although trial experience in this group is limited.

Aims: To assess the safety and efficacy of DE 150 mg qd in patients with moderate RI undergoing THR/TKR in clinical practice.

Methods: This open, prospective, uncontrolled, observational cohort study included adult patients with moderate RI (creatinine clearance [CrCL] 30–50 mL min⁻¹) in 7 EU countries. In accordance with the European label, patients were to receive DE 75 mg 1–4 h after surgery and 150 mg qd from day 2 to day 10 (TKR) or day 35 (THR). Primary safety outcome was major bleeding events (MBEs) and efficacy outcome was symptomatic VTE and all-cause mortality, from the 1st dose until 24 h after the last intake of DE. Ethics committee approval and patients' informed consent were obtained ahead of the studies.

Results: Four hundred and twenty-eight patients with moderate RI received DE (221 THR; 207 TKR; median treatment duration 31 and 28 days, respectively; median CrCL 43.4 [30–49.9] mL min⁻¹; median age 80 [32–96] years). Ten MBEs occurred in 9 patients overall (2.1% of patients with MBE; 95% confidence interval [CI] 1.0–4.0); none were fatal or involved a critical organ. MBE rates in the THR/TKR groups were 1.8%/2.4%, respectively. Symptomatic VTE and all-cause mortality occurred in 3 patients overall (0.7%; 95% CI 0.1–2.0): 2 in the THR group (0.9%) and 1 in the TKR group (0.5%).

Conclusion: The real-world data obtained in this 1st observational study of DE 150 mg qd in an elderly, fragile population, complement previous clinical trial results indicating that DE 150 mg qd is an appropriate dose for patients with moderate RI undergoing THR/TKR.

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OR284

Efficacy of rivaroxaban for prevention of venous thromboembolism after knee arthroscopy: a randomized double-blind trial (ERIKA study)

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Background: Without thromboprophylaxis knee arthroscopy (KA) carries a definite risk of venous thromboembolism (VTE). Despite the huge number of KA procedures worldwide per year, the latest ACCP guidelines do not recommend routine prophylaxis in this setting. Efficacy of rivaroxaban for VTE prevention after major orthopaedic surgery is well established. No randomized clinical trials employing rivaroxaban for VTE prevention after KA are available.

Aims: To assess efficacy and safety of rivaroxaban for VTE prevention after KA.

Methods: Multicentre, randomized, double-blind, placebo-controlled trial. Written informed consent was obtained from all patients. Patients were randomized to rivaroxaban (10 mg od) or placebo for 7 days. All patients underwent bilateral whole-leg color-coded Doppler ultrasonography at day 7 (+1), or earlier in case of symptoms/signs of VTE. A 3-month follow-up visit was planned. Primary efficacy outcome (PEO) was the combined incidence of all-cause mortality, symptomatic VTE and asymptomatic proximal deep-vein thrombosis. Primary safety outcome was major bleeding.

Results: Overall 241 patients were randomized (122 rivaroxaban, 119 placebo). The 3-month cumulative incidence of PEO was 0.8% in the rivaroxaban group vs. 6.1% in the placebo group (ARR: -5.3%; 95% CI -11.3 to -0.4; crude RR 0.13; 95% CI 0.02-1.1; *P* = 0.03; NNT = 19). No major bleeding observed. Minor peri-surgical site bleeding incidence 3.3% in the rivaroxaban group vs. 5.3% in the placebo group (ARR: -2.0%; 95% CI -8.0 to -3.7; crude RR 0.7; 95% CI 0.2-2.3; *P* = 0.53; NNH = 52).

Conclusion: Oral rivaroxaban 10 mg od for 7 days is effective and safe for VTE prevention in patients undergoing KA. (ClinicalTrials.gov Id: NCT01629381).

Disclosure of Interest: G. Camporese Grant/Research Support from: Bayer, Daiichi Sankyo, Aspen, Alfa Wassermann, Consultant for: Daiichi Sankyo, Bayer, E. Bernardi Consultant for: Bayer, F. Noventa: None declared.

OR285

Assays to measure direct oral anticoagulants (DOAC): data from UK Neqas multicentre studies

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Background: Direct oral anticoagulants (DOACs) Dabigatran, Rivaroxaban and Apixaban are increasingly used in preference to Coumadin, and routine monitoring is not required. However, there are circumstances in which specific DOAC assays are indicated, and many laboratories are establishing or modifying assays to measure DOACs. Information on between-centre precision for these assays is limited.

Aims: We describe here a multicentre study assessing performance of assays for DOACs.

Methods: Three lyophilised plasma samples were prepared for each DOAC, 1 containing no drug, and 2 spiked with different concentrations of drug, kindly provided by the manufacturers. UK NEQAS cen-

tres were asked to perform their routine assay for each DOAC. Median, coefficient of variation (CV) and range were determined for each method and reagent group.

Results: Ten different methods were employed to assay Dabigatran and Rivaroxaban, with 6 methods reported for Apixaban assay. 79% of centres used a single source of calibrator for Dabigatran assays. For Rivaroxaban and Apixaban, calibrator source was split between Stago, Technoclone and Hyphen. Medians (ranges) for the 3 Dabigatran samples were 0 ng mL⁻¹ (0-40), 34 ng mL⁻¹ (5-75) and 158 ng mL⁻¹ (80-250) respectively. High CVs were observed for the two samples with lower levels of Dabigatran, at or below limits of detection for these assays. Medians (ranges) for the 3 Rivaroxaban samples were 8 ng mL⁻¹ (0-102), 37 ng mL⁻¹ (12.9-80) and 140 ng mL⁻¹ (94-473) respectively. For Apixaban, medians (ranges) were at 4 ng mL⁻¹ (0-60), 45 ng mL⁻¹ (21-70) and 180 ng mL⁻¹ (131-221) respectively. Between-method variability was not marked, but large between-centre differences were observed.

Conclusion: Despite variability in methods and calibrators, measurement of therapeutic levels of DOACs showed generally good precision in this study; however, marked variation in results reported by individual laboratories highlights the need for quality assurance of these assays.

Disclosure of Interest: None declared.

Contact System I

OR286

Arterial thrombosis is accelerated in histidine-rich glycoprotein deficient mice

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Background: Polyanions, such as nucleic acids, have been identified as potent activators of the contact system. However, natural mechanisms to limit the contact system have not been elucidated. Previously, we showed that the plasma protein, histidine-rich glycoprotein (HRG) binds factor (F) XIIa with high affinity and attenuates its capacity to propagate coagulation.

Aims: To test the hypothesis that HRG attenuates nucleic acid driven arterial thrombosis in a murine model.

Methods: The effects of HRG on DNA- and RNA-mediated activation of coagulation were assessed in human and mouse plasma and the time to occlusion (TTO) after FeCl₃-induced carotid artery injury in HRG-deficient mice was compared with that in wild-type mice. Mice were also injected with DNase or RNase, or FXII or FVII was selectively depleted using antisense oligonucleotides prior to FeCl₃-injury. Blood loss after tail amputation was used to assess hemostasis. Thrombi were subjected to immunohistochemical analysis to detect HRG, fibrin and platelets.

Results: RNA- and DNA-mediated activation of coagulation was accelerated in plasma from HRG-deficient mice, and returned to normal with HRG addition. The TTO after FeCl₃-induced injury was shorter in HRG-deficient mice than wild-type mice (7.2 ± 2.6 and > 30 min, respectively; *P* < 0.001); a difference abrogated by administration of human HRG. HRG was detected in thrombi and localized with platelets and fibrin. Neither DNase nor selective depletion of

FVII had an impact on thrombosis. In contrast, RNase or selective depletion of FXII abrogated FeCl₃-induced occlusion, indicating that in this model, thrombosis is triggered by RNA in a FXII-dependent fashion. Blood loss in HRG-deficient and wild-type mice was similar.

Conclusion: HRG is a potent inhibitor of nucleic acid-driven coagulation, suggesting that it is a regulator of the intrinsic pathway of coagulation.

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Phosphate polymers and factor XI activation

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Background: Factor XI (fXI) is the zymogen of a protease (fXIa) that contributes to thrombosis and inflammation. Phosphate polymers such as inorganic phosphate (polyP), DNA and RNA enhance fXI activation. PolyP-enhanced fXI activation by thrombin (IIa), factor XIIa (fXIIa) or autoactivation requires Zn²⁺ ions, and anion-binding sites on the fXI A3 (ABS1) and catalytic (ABS2) domains.

Aims: To compare fXI activation by IIa, fXIIa and autoactivation in the presence of polyP, DNA and RNA; and determine the importance of ABS1, ABS2, and Zn²⁺ to the reactions.

Methods: The effects of polyP, leukocyte DNA or liver RNA on fXI activation were studied with chromogenic and thrombin generation assays. Wild type fXI (fXI-WT) was compared to fXI lacking ABS1 (fXI-ABS1) or ABS2 (fXI-ABS2).

Results: PolyP, DNA and RNA had minimal effect on fXI (30 nM) activation by IIa, fXIIa or autoactivation in the absence of Zn²⁺. With 10 μM Zn²⁺ and optimal DNA (5 μg mL⁻¹) or RNA (1 μg mL⁻¹), complete activation was induced within 30–60 min. In comparison, polyP (200 μM) was a weak inducer of autoactivation with 10 μM Zn²⁺ under physiologic [NaCl]. IIa increased the rate of fXI activation ~6-fold with DNA and 2-fold with RNA. fXIIa increased activation ~2-fold with DNA, and had little effect with RNA. In comparison, IIa increased activation ~20-fold with polyP. With DNA and RNA, fXI-ABS2 failed to autoactivate, while fXI-ABS1 was activated ~10-fold slower than fXI-WT. Addition of DNA to normal plasma initiated thrombin generation that was entirely dependent on fXI, and partially dependent on fXII.

Conclusion: At physiologic Zn²⁺ and NaCl concentrations DNA and RNA are more potent enhancers of fXI activation than polyP. In contrast to polyP, DNA and RNA primarily enhanced autoactivation in a reaction requiring fXI ABS2, with some contribution of ABS1. The results support the notion that fXI ABSs mediate interactions with polyanions, and are critical for the recruitment of fXI for thrombotic and inflammatory processes. (1994).

Disclosure of Interest: None declared.

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Factor XII activation by activated factor XI

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Background: Phosphate polymers such as inorganic phosphate (polyP), DNA and RNA in cell-free forms may induce contact activation at sites of vascular injury, inflammation or infection. During contact activation, factor XII (fXII) is converted to the protease fXIIa by kallikrein and by autoactivation. fXIIa then contributes to thrombin generation by converting factor XI (fXI) to fXIa, a protease structurally similar to kallikrein. There is evidence that fXIa, like kallikrein, activates fXII, but this has not been studied in detail.

Aims: To study the effects of DNA, RNA and Zn²⁺ ions on fXII activation by kallikrein, fXIa and autoactivation.

Methods: fXII activation was studied in the presence of leukocyte DNA or mouse liver RNA, and different Zn²⁺ concentrations using chromogenic assays and SDS-PAGE.

Results: fXII (200 nM) autoactivation was minimal in the presence of DNA (5 μg mL⁻¹) or RNA (5 μg mL⁻¹) in the absence of Zn²⁺, but was markedly enhanced by 10–25 μM Zn²⁺. RNA gave a slightly larger effect than DNA. In the absence of DNA or RNA, fXII activation by fXIa (2 nM active sites) was ~2-fold faster than activation by kallikrein (2 nM). In the presence of 10 μM Zn²⁺, DNA or RNA enhanced fXII activation by fXIa ~25-fold, and by kallikrein ~2-fold.

Conclusion: The homologs fXIa and kallikrein activate fXII similarly in the absence of polyanions. DNA or RNA have a larger effect (~10-fold) on Zn²⁺-dependent fXII activation by fXIa than by kallikrein. In classic contact activation, fXIIa converts fXI to fXIa, which then contributes to thrombin generation by activating fIX. However, fXI is activated independently of fXIIa during coagulation. The results raise the possibility that fXIa generated during tissue factor-initiated thrombin generation may activate the contact system, and support the hypothesis that fXI/XIa forms a bidirectional interface between thrombin generation and contact activation, allowing the two systems to influence each other.

Disclosure of Interest: None declared.

OR289

An allosteric disulfide bond is involved in enhanced activation of factor XI by protein disulfide isomerase

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Background: Protein disulfide isomerase (PDI) was shown to reduce factor XI (FXI) and to enhance its ability to cleave its chromogenic substrate. Three disulfide bonds in FXI (118-147, 362-482 and 321-321) were shown to be involved in this augmented activation.

Aims: To characterize the mechanisms by which PDI enhances FXI activity.

Methods: The ability of PDI-reduced FXI to cleave its chromogenic substrate, S2366 or its physiologic substrate, factor IX (FIX) were measured after thrombin activation. The rate of conversion of reduced FXI to FXIa was assessed by immunoblotting. To assess the involvement of each of the three disulfide bonds in FXI enhanced activation, FXI harboring mutations that disrupt these bonds (C147S, C482S or C321S) or wild type (WT) FXI were expressed in baby hamster kidney

(BHK) cells. Antigenicity of secreted FXI was measured by ELISA and its activity was assessed using a chromogenic substrate. The effect of disulfide bond reduction on FXI structure was analyzed by molecular dynamics (MD).

Results: FXI reduction by PDI enhanced cleavage of both its chromogenic and its physiologic substrates. Incubation with PDI increased the conversion of FXI to FXIa as shown by immunoblotting. C147S-FXI expressed in BHK cells was not secreted to the medium. C321S-FXI and C482S-FXI were secreted in smaller amounts than WT-FXI. WT and C321S-FXI exhibited similar rates of chromogenic substrate cleavage, while C482S-FXI cleaved it faster than WT. Configuration analysis of the disulfide bonds in FXI revealed that the 362-482 bond has an allosteric configuration. MD analysis showed that disruption of the 362-482 bond gave rise to a broader thrombin binding site in FXI.

Conclusion: Reduction of FXI by PDI enhances its ability to cleave its physiologic substrate, FIX, probably by faster conversion of FXI to FXIa. The allosteric disulfide bond 362-482 is involved in the increased FXI activation upon its reduction possibly by increasing thrombin accessibility to FXI.

Disclosure of Interest: None declared.

OR290

DNA and RNA activate the contact pathway of coagulation by promoting reciprocal activation of factor XII and prekallikrein

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Background: The contact system, which consists of factor XII (FXII), prekallikrein (PK), and high-molecular weight kininogen (HK), initiates the intrinsic pathway of coagulation. Studies suggest that DNA and RNA activate the contact system by serving as physiological surfaces onto which the contact factors assemble.

Aims: To determine the extent to which DNA and RNA activate the contact system, and to identify the mechanism.

Methods: Thrombin generation was quantified in platelet poor plasma in the absence or presence of 40 µg mL⁻¹ DNA or RNA. In buffer systems, the effect of DNA or RNA on (a) FXII activation in the absence or presence of PK and/or HK, and (b) PK activation in the absence or presence of FXIIa and/or HK was determined using chromogenic assays.

Results: Nucleic acids enhanced thrombin generation in platelet poor plasma; DNA and RNA significantly ($P < 0.05$) increased the endogenous thrombin potential by 19% and 14% (from 3124 ± 283 nM*min to 3715 ± 138 and 3558 ± 68 nM*min), shortened the lag phase by 80% and 35% (from 27 ± 5 min to 5 ± 0.5 and 18 ± 2 min), and increased peak thrombin concentration by 2- and 1.5-fold (from 71.5 ± 17 nM to 146 ± 7 and 112 ± 11 nM), respectively. In a purified system, DNA augmented FXII autoactivation by 3-fold, whereas RNA had no effect. Both DNA and RNA promoted FXII activation by 7-fold in the presence of PK, and by 11-fold in the presence of PK and HK. DNA or RNA alone enhanced FXIIa-mediated activation of PK by 2-fold, whereas in the presence of HK, they augmented activation by 10- and 8-fold, respectively. Therefore, nucleic acids promote contact activation.

Conclusion: DNA and RNA promote thrombin generation by augmenting reciprocal activation of FXII and PK in the absence or presence of HK, likely by serving as a template onto which the contact factors assemble. This phenomenon may contribute to thrombosis at sites of tissue damage.

Disclosure of Interest: None declared.

Cancer and thrombosis – clinical – III

OR291

Thrombotic burden and the risk of subsequent manifest cancer

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Background: Whether the extension of initial thrombosis and the persistence of residual thrombosis predict the development of subsequent cancer in patients with deep vein thrombosis (DVT) is unknown.

Aims: To correlate the extension of initial thrombosis and the persistence of residual thrombosis with the development of cancer in patients with proximal DVT.

Methods: In 1081 consecutive cancer-free patients who were followed up for up to three years, the extension of thrombosis and the simultaneous presence of PE were recorded, as was the ultrasound assessment of residual vein thrombosis (RVT), defined as the incompressibility of at least 4 mm in the transverse section after three months.

Results: Subsequent cancer developed in 15/458 (3.3%) patients with extensive thrombosis (involvement of both the common femoral and the popliteal vein) and in 35/623 (5.6%) with more limited DVT; in 3/154 (1.9%) patients with and in 47/927 (5.1%) without simultaneous PE. After adjusting for age, sex, previous thromboembolism and the modality of DVT presentation (idiopathic vs. secondary) the OR of subsequent cancer in patients with extensive as compared to those with more limited thrombosis was 0.57 (95% CI, 0.31–1.1); and was 0.31 (95% CI, 0.09–1.1) in patients with as compared to those without PE. RVT was detected in 510 of the 985 cancer-free patients (51.8%) in whom this determination could be done. Subsequent cancer developed in 21 (4.1%) patients with and in 8 (1.7%) without RVT. The adjusted OR of subsequent cancer in patients with as compared to those without RVT was 2.6 (95% CI, 1.1–6.1). Patients with unprovoked DVT and RVT had the highest risk of developing subsequent cancer.

Conclusion: In cancer-free patients with proximal DVT, the development of subsequent cancer is not correlated with the extension of initial thrombosis, nor is it with the simultaneous presence of PE. In patients who do not develop cancer in the first three months, the assessment of RVT is an independent predictor of subsequent overt malignancy.

Disclosure of Interest: None declared.

OR292

Recurrent venous thromboembolism and mortality in cancer patients with upper extremity deep vein thrombosis

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Background: Cancer is a common risk factor for upper extremity deep vein thrombosis (UEDVT). Data on the risk of recurrent venous thromboembolism (VTE) and mortality of cancer patients after a diagnosis of UEDVT are scarce.

Aims: To evaluate the risk of recurrent VTE in cancer patients after a diagnosis of UEDVT, and to assess survival vs. cancer patients without UEDVT.

Methods: Follow-up data were collected from patients with suspected UEDVT who were enrolled from January 2010 to June 2012 in an international diagnostic management study (Kleinjan et al., Ann Intern Med, 2014). Between August 2014 and January 2015, patients were interviewed by telephone and questioned about recurrent VTE and mortality. We compared the risk of recurrent VTE between cancer and non-cancer patients with UEDVT and the risk of mortality between cancer patients with and without confirmed UEDVT using a Cox proportional hazards model.

Results: Out of 177 patients, UEDVT was diagnosed in 42 cancer and 62 non-cancer patients. Thrombosis was ruled out in 73 cancer patients. Follow-up was completed for 174 of 177 patients (98.3%) with a median follow-up of 3.0 years (IQR 2.2–3.8). Recurrent VTE after UEDVT occurred approximately twice as often in patients with cancer (12.2%) relative to those without cancer (6.5%; HR 1.68, 95% CI 0.51–5.55). Of the cancer patients with UEDVT 51.2% died vs. 43.1% of the cancer patients without UEDVT (HR 1.47, 95% CI 0.84–2.58); when adjusted for age and presence of metastases, the HR was 1.26 (95% CI 0.69–2.30).

Conclusion: Cancer patients with UEDVT seem to have a higher risk of recurrent VTE than non-cancer patients. A diagnosis of UEDVT does not appear to be associated with worse survival in cancer

patients, however, this may be due to the small number of patients in this study.

Disclosure of Interest: None declared.

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Interrelation between cancer and myocardial infarction in the general population

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Background: The association between venous thromboembolism (VTE) and cancer is well known, but there is limited data on the association between cancer and myocardial infarction (MI). Results from a registry-based study suggest that cancer is associated with increased risk of MI. Data from registry studies should, however, be interpreted with caution due to lack of validation of outcomes and ability to adjust for confounders.

Aims: To study the association between cancer and MI in a large prospective cohort recruited from the general population.

Methods: Subjects ($n = 28\,740$) without a history of MI and cancer were included from the Tromsø Study and followed from baseline to the date of cancer, death, migration or study end. Cox regression models with age as time scale were used to calculate hazard ratios (HR) for cancer-related MI adjusted for sex, BMI, diabetes, smoking, blood pressure, HDL-cholesterol, physical activity and education. The regional ethical committee approved the study and all participants gave their informed written consent.

Results: There were 1858 incident MIs and 2677 incident cancers during a median follow-up of 15.7 years. Subjects with MI had increased risk of subsequent cancer compared with subjects without MI (HR 1.60, 95% CI 1.15–2.21 in women and 1.28, 95% CI 1.02–1.61 in men). The risk was highest the first 6 months after the MI (HR 3.11, 95% CI 1.48–6.61 in women and 1.74, 95% CI 0.90–3.36 in men), and remained elevated after one year in women (HR 1.58, 95% CI, 1.00–2.49), but not in men (HR 1.34, 95% CI, 0.97–1.85). After 5 year was the association not longer significant (HR 1.39; 95% CI, 0.76–2.53 in women and 1.24, 95% CI 0.86–1.79 in men). Likewise, cancer patients had increased risk of MI the first 6 months after the cancer diagnosis (HR 2.11, 95% CI 1.05–4.25 in women and 1.79, 95% CI 1.07–3.00 in men), but declined rapidly thereafter and were no longer significant.

Conclusion: Our results imply that cancer and MI are interrelated, and that MI may be the first sign of occult cancer.

Disclosure of Interest: None declared.

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Epidemiology of first venous thromboembolism in patients with active cancer

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Background: Population studies on the epidemiology of venous thromboembolism (VTE) in patients with active cancer are limited. The frequency of cancer in VTE cohorts has been well described but there are no published data for the incidence rates (IR) for VTE among patients with active cancer.

Aims: n/a.

Methods: A population based observational cohort study was undertaken to estimate the incidence of first VTE in patients with active cancer. The source population consisted of all patients in the UK Clinical Practice Research Datalink, with additional linked information on hospitalisations and cause of death. The 90 days before and after a cancer-related clinical diagnosis or therapy defined the period of an active cancer (at-risk period). Between 2001 and 2011 all first VTE events during the at-risk period were identified using a validated algorithm. IR of first cancer-associated VTE were provided by age, gender and VTE type, deep vein thrombosis (DVT) and pulmonary embolism (PE).

Results: In a total of 139,467 patients with cancer contributing 112,738 active cancer-associated person-years of observation, 6592 incident VTEs, 46.3% DVTs and 53.7% PE with or without DVT, were observed. The mean age of VTE was 68.8 years. The most common types of cancer among VTE cases were prostate cancer in men (18.2%), breast cancer in women (16.7%), lung cancer (14.7%) and colon cancer (13.5%). IRs of VTE were 5.8 (CI 5.7–6.0) per 100 person years, 2.7 for DVT and 3.1 for PE. The IR of first VTE was highest in the elderly population, and peaked for men in the 6th decade (6.3 per 100 person-years) and for women in their 8th decade (7.6 per 100 person-years). Similar increases with age were seen for first DVT and first PE.

Conclusion: VTE in patients with active cancer is common. Efforts are needed to reduce the high incidence.

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The risks and benefits of thromboprophylaxis (TP) among hospitalized medically ill cancer patients

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Background: Pharmacological TP for all cancer patients hospitalized for acute medical illness is considered standard practice and strongly

recommended in clinical guidelines. However, cancer patient-specific data are lacking to help estimate net risk-benefit of TP.

Aims: To assess the incidence of major bleeding (MB) and venous thromboembolism (VTE) among hospitalized medically-ill cancer patients.

Methods: Retrospective cohort study of consecutive hospitalized medically-ill cancer patients admitted between January and June 2010 in three academic centers. The primary efficacy and safety outcome measures were symptomatic VTE (SSC recommended definition) and MB (ISTH definition). Anticoagulant TP was defined as prophylactic doses of low-molecular-weight-heparin or unfractionated heparin.

Results: A total of 2030 patients were included and 217 (11%) were deemed to have a contraindication to anticoagulant TP. Mean age was 63 years old and 51% were male. The most frequent tumor types were non-small cell lung carcinoma (13%) and lymphoma (11%). The median length of stay in hospital was 7 days (range 0–131). The most frequent reasons for admission were infections (8%) and chemotherapy delivery (10%). Of the 1813 patients without a contraindication, 734 (41%) patients received TP during their hospitalization. Among these patients, the rate of VTE was 3% (95% CI 1.9–4.5), compared with 3.4% (95% CI 2.4–4.7) in those who did not receive TP. The rate of MB was 1.9% (95% CI: 1.1–3.2) and 1.2% (95% CI: 0.6–2.1), respectively. Among those given TP, 71% had at least one additional risk factor for VTE and 58% had one or more risk factors for bleeding. Among those not given TP, 63% and 47% had risk factors for VTE and bleeding, respectively.

Conclusion: Rates of VTE were similar for medically-ill cancer patients receiving anticoagulant TP or not. The risk of MB was low in both groups. Decision to provide TP does not appear to be based on risk-benefit assessment.

Disclosure of Interest: None declared.

Factor VIII and IX

OR296

A unique exon specific U1snRNA rescues different haemophilia B – causing splicing-defective factor IX variants in mice

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Background: Exon-skipping causing mutations, frequent causes of human disease, have been corrected by engineered spliceosomal U1snRNA targeting specific intronic sequences downstream of donor splice sites (Exon Specific U1, ExSpeU1). However, ExSpeU1s have been poorly investigated *in vivo* and never in coagulation factor defects, which benefit from tiny increases of functional protein levels. We have identified an ExSpeU1 (ExSpeU1sh9) able to correct *in vitro* a panel of F9 exon 5 exon-skipping mutations, which affects several severe Haemophilia B (HB) patients.

Aims: To assess in mice the ExSpeU1sh9-mediated rescue of the F9 c.520-2A>G (IVS5-2A>C) and c.392-8T>G (IVS5-8T>G) mutations at donor (5'ss) and acceptor splice sites.

Methods: HB mouse models, created by hydrodynamic injection of plasmids for the human hFIX splicing-competent cassettes (pF9IVS5-2c; pFIVS4-8 g) in wt BALB/c mice, were co-injected with vectors for ExSpeU1sh9, U1wt or the first generation U1F9wt targeting the F9IVS5-5'ss. hFIX expression was evaluated by human-specific RT-PCR, ELISA and Western Blotting.

Results: Mice injected with both pF9IVS5 variants expressed trace plasma levels of a truncated FIX arising from the exon 5-skipped F9 transcript. Co-delivery with a 1.5 molar excess of pExSpeU1sh9 resulted in remarkable increase of plasma hFIX (IVS2-2c,

450 ± 89 ng mL⁻¹; IVS5-8 g, 920 ± 300 ng mL⁻¹; ~31% and ~64% of the pF9IVS5wt, respectively) and, most importantly, restoration of the full-length hFIX protein form. This was consistent with an appreciable rescue of correct hFIX transcripts (IVS2-2c, 85 ± 5%; IVS5-8 g, 72 ± 4%) in liver of both HB models. Comparable results were obtained with the pU1F9wt whereas the pU1wt was ineffective, to indicate a specific ExSpeU1-mediated rescue.

Conclusion: We provide the first *in vivo* proof-of-principle that a unique ExSpeU1 efficiently rescues different exon-skipping mutations causing HB, which further strengthens the therapeutic potential of this approach for coagulation disorders and other genetic diseases.

Disclosure of Interest: None declared.

OR297

Suppression of 'leaky' nonsense mutations by ribosome readthrough accounts for residual factor IX levels in haemophilia B patients

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Background: Nonsense mutations, relatively frequent in Haemophilia (> 10%), are considered 'null mutations'. However, they have been found also in moderate/mild Haemophiliacs and, as compared with large gene deletions, are associated with lower risk for developing inhibitors. This observation point to the presence of residual expression levels arising from 'ribosome readthrough' over nonsense triplets, as we have shown in a very small cohort of Haemophilia B (HB) patients. Drugs inducing readthrough such as aminoglycosides are proposed as potential therapy.

Aims: To investigate residual levels of factor IX (FIX) produced by an extended panel of HB nonsense mutations.

Methods: Expression of nonsense FIX variants in HEK293 cells, evaluation of intracellular and secreted FIX levels (ELISA, Western Blotting, fluorogenic functional assays).

Results: We investigated nine F9 mutations (R75X, L103X, R162X, R294X, R298X, Y330X, Q370X, R379X, R384X) associated to severe/moderate HB. Appreciable levels of secreted FIX were detected for the R162X, R294X, R298X, Y330X mutants, with a large predominance of truncated forms. Truncated forms for Q370X, R379X and R384X were observed in cell lysates only, which suggests misfolding. Noticeably, the full-length FIX form was appreciable for the R162X, R294X and R298X and indicated the occurrence of spontaneous readthrough, which was significantly increased by the use of aminoglycosides. Moreover, aminoglycosides promoted the synthesis of full-length FIX in the presence of the R75X, Y330X, Q370X, R379X, R384X mutations, not undergoing appreciable spontaneous readthrough. Preliminary functional assays revealed a residual activity for the R162X mutant.

Conclusion: These data demonstrate that nonsense mutations can be associated to residual FIX levels through a mechanism of 'productive' readthrough. The identification of 'leaky' nonsense mutations, and thus of patients with trace FIX levels and potentially 'high responders' to readthrough-inducing drugs, might help diagnosis and treatment.

Disclosure of Interest: None declared.

OR298

F8 and F9 gene variants, CADD scores, and hemophilia severity: in the my life, our future genotyping project

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Background: My Life, Our Future (MLOF), a partnership of PSBC, ATHN, NHF, and Biogen Idec, is conducting U.S.-wide hemophilia genetic testing and building a research repository. This effort will identify genetic variants of unknown function, which pose challenges to interpretation.

Aims: To study a genomic computational tool, the scaled-CADD score (C score), which integrates diverse annotations into a single measure, for prediction of functional impact of F8 and F9 gene variants in MLOF.

Methods: Initial screening for DNA variants was by targeted F8 and F9 next generation sequencing (NGS). Candidates were independently validated in the PSBC CLIA-certified laboratory prior to clinical reporting. In 1052 patients, we obtained a C score for all DNA variants identified by NGS (except large structural variants). We analyzed scores with regard to gender, disease, baseline factor level, clinical action (reporting), and frequency.

Results: NGS identified one or more non-structural gene variants in 99% of patients. There were 404 discrete variants (299 in F8; 105 in F9). Most (96%) gene variants were rare, found in < 1% of patients. C scores were higher ($P < 1 \times 10^{-17}$) for clinically reported variants (25.9 ± 7.7 in F8; 27.3 ± 7.5 in F9) than unreported variants (7.7 ± 7.5 in F8; 3.4 ± 4.3 in F9). Reported variant C scores averaged > 20 regardless of severity of hemophilia A [mild (22.9 ± 5.3), moderate (25.0 ± 5.3), severe (27.6 ± 7.8)] or hemophilia B [mild (22.2 ± 8.9), moderate (26.6 ± 5.2), severe (28.9 ± 7.3)]. However, 3.8% of reported variants had C scores < 10 and 6.6% of unreported variants had C scores > 20.

Conclusion: Overall, C score correlated well with independent assessments of F8 and F9 gene variant function, with higher scores generally corresponding with deleterious variants. We propose the C score as a useful and evolving research tool, but caution against over-interpretation of individual scores until further studies can be done.

Disclosure of Interest: None declared.

OR299

CB-FIX: an improved second generation FIX drug candidate

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Background: Wild type (wt), recombinant human FIX (e.g., Bene-FIX[®]) is routinely used as enzyme replacement therapy to treat hemophilia B patients. Due to a relatively short duration of action, however, prophylactic use of wt FIX to prevent spontaneous bleeding requires very frequent dosing (i.e., 2–3 times/week).

Aims: To use structure-based rational design and molecular modeling to create a novel variant of human FIX (CB-FIX) that exhibits a significantly longer duration of action than wt FIX and allows less frequent prophylactic dosing.

Methods: We measured the procoagulant activity (i.e., catalytic efficiency for activation of FX to FXa in the presence of FVIIIa) of Bene-

FIX[®] and CB-FIX, affinity for FVIIIa, rate of inhibition by ATIII, efficacy and duration of action for inhibition of bleeding ('tail clip' model) in FIX^{-/-} mice, and duration of 'aPTT correction' in non-bleeding FIX^{-/-} mice. Finally, we performed single dose escalation toxicity/safety studies using wild type mice.

Results: Compared with BeneFIX[®], CB-FIX exhibited approximately 2.8-fold enhanced procoagulant activity, approximately 8-fold greater affinity for FVIIIa, and more than 20-fold enhanced resistance to inhibition by ATIII. *In vivo*, CB-FIX exhibited approximately 4-fold enhanced efficacy in inhibiting blood loss ('tail clip' model) and an approximately 8-fold prolonged duration of action in both this acute bleeding model and also for correction of aPTT activity in non-bleeding hemophilia B (FIX^{-/-}) mice. Finally, comparing data from murine efficacy and toxicity/safety studies suggested that CB-FIX also exhibited a modestly enhanced therapeutic index compared with BeneFIX[®].

Conclusion: Based on these improved *in vitro* and *in vivo* properties, CB-FIX appears to represent a novel second generation FIX variant that is significantly differentiated from both BeneFIX[®] and other advanced second generation FIX variants.

Disclosure of Interest: E. Madison Employee of: Catalyst Biosciences, Inc., C. Thanos Employee of: Catalyst Biosciences, Inc., G. Blouse Employee of: Catalyst Biosciences, Inc., M. Fox Employee of: Catalyst Biosciences, Inc., R. Overbeck Employee of: Catalyst Biosciences, Inc., H. Ly Employee of: Catalyst Biosciences, Inc., K. McCluskie Employee of: Catalyst Biosciences, Inc., D. Sloane Employee of: Catalyst Biosciences, Inc.

OR300

Correction of aberrant splicing causing haemophilia B through the combination of compensatory U1snRNAs and antisense oligonucleotides

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Background: Modulation of mRNA splicing is emerging as a promising approach for the therapy of genetic diseases, with advantages (maintenance of gene regulation in physiological tissue only; small therapeutic transgene) over replacement gene therapy. Mutations affecting splicing are relatively frequent in severe coagulation factor disorders, which would benefit even from tiny increase of functional protein levels.

Aims: To exploit the knowledge of aberrant splicing mechanisms caused by a panel of Haemophilia B (HB)-causing mutations at the donor splice site (5'ss) of F9 exon 2 to develop a new potential therapeutic approach.

Methods: Expression of splicing-competent FIX cDNA cassettes harboring c.252 + 3G>C, c.252 + 5G>A, c.252 + 5G>C, c.252 + 5G>T and c.252 + 6T>C mutations in mammalian cells. Creation of modified U1snRNAs and design of antisense oligonucleotide (AON). Evaluation of FIX mRNA (RT-PCR).

Results: Expression studies demonstrated that all mutations lead to a deleted F9 mRNA form (F9del) due to the usage of an exonic cryptic 5'ss, which is down-regulated by a highly conserved exonic splicing silencer (ESS). Interestingly, HB-causing missense changes at the ESS also promote synthesis of the F9del aberrant form.

Antisense oligonucleotides masking the cryptic 5'ss failed to re-direct usage of the authentic 5'ss in the presence of 5'ss mutations, and promoted exon 2 skipping. On the other hand, small nuclear RNA U1 (U1snRNA) variants with increased complementarity to the defective 5'ss, a strategy exploited to rescue splicing, were also ineffective. Noticeably, the combination of the modified U1snRNAs with AONs

produced appreciable amounts of correctly spliced transcripts (from 0% to 20–40%) for virtually all 5'ss mutants.

Conclusion: For the first time we demonstrated that 5'ss mutations inducing usage of a cryptic 5'ss, not uncommon in human disease, can be effectively counteracted by the synergistic combination of compensatory U1snRNAs and antisense molecules, thus proposing a novel RNA-based therapeutic approach.

Disclosure of Interest: None declared.

Blood coagulation tests – I

OR301

Simultaneous measurement of thrombin generation and fibrin formation in whole blood applying continuous flow is indicative for the amount of blood loss during/after cardiothoracic surgery

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Background: Thrombin generation (TG) and fibrin formation (FF) do not always go hand in hand as factor XIIa independently of thrombin influences the crosslinking of fibrin. Flow has been shown to profoundly influence TG and FF.

Aims: The validation of an assay that is able to simultaneously measure TG and FF in whole blood under conditions of flow.

Methods: We have redesigned an air-bearing rheometer, rendering it sensitive to measure FF based on changes in viscosity. Introduction of a fluorescence detector allowed the measurement of TG based on the conversion of a thrombin-sensitive substrate, while applying laminar flow within the range of 100–1200 s⁻¹.

Results: For all variables the intra-/inter-assay variation was below 15% in platelet-poor plasma (PPP) and whole blood. For the endogenous thrombin potential (ETP), peak and maximum clot strength (viscosity) we demonstrated an inverse relationship with increasing flow rates in PPP. The addition of increasing concentrations of fibrinogen to defibrinated plasma resulted in an increase in ETP, peak and maximum clot strength. In 70 patients undergoing cardiothoracic surgery, TG parameters in blood samples taken pre-bypass correlated with traditional calibrated automated thrombinography parameters, whereas FF parameters correlated with fibrinogen content and rotational thromboelastometry parameters. Upon dividing patients into two groups based on the median clot strength, a significant difference in perioperative/total blood loss was established.

Conclusion: We clinically validated a method capable of simultaneously measuring TG and FF in plasma/whole blood under continuous flow, rendering our method one step closer to physiology. Our method was sensitive enough to detect differences in fibrinogen content and revealed an effect of different flow rates on both TG and FF. Importantly, in contrast to the traditionally used methods, our test was indicative for the amount of blood loss during/after cardiothoracic surgery.

Disclosure of Interest: None declared.

OR302

A novel substrate for use in a point of care thrombin generation assay

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Background: The uses of small molecule substrates of thrombin, such as S2238, in whole blood are limited because of their poor specificity and inability to distinguish thrombin inhibited by alpha-2-macroglobulin (a2M).

Aims: To develop a thrombin substrate for use in whole blood.

Methods: When thrombin cleavable sequences, such as LVPRGVNL, were inserted between two coral proteins, cleavage, monitored by loss of fluorescence resonance energy transfer, was slow. Sequences predicted to form an anti-parallel β -sheet added to both sides of the cleavage sequence increased the k_{cat}/K_m for thrombin by 29,000-fold (protein termed T13). The K_m of T13 for human thrombin was 5.5 μ M; activated protein C cleaved T13 with 82,000-fold lower efficiency.

Results: Anion binding exosite (ABE) II RA-thrombin cleaved T13 as well as it cleaved S2238; gamma-thrombin did not cleave T13. ABE I-directed aptamer HD1 inhibited thrombin cleavage of T13, but ABE-II aptamer HD22 did not. These findings suggest an interaction between T13 and ABE-I of thrombin. Soluble thrombomodulin increased the K_m of thrombin for T13 by 5-fold, and decreased the k_{cat} 5-fold. Addition of a2M inhibited the ability of thrombin to clot plasma and to cleave T13 but not S2238.

When unanticoagulated blood, obtained from a finger-prick from a healthy volunteer, was added to T13 in a polystyrene vessel, cleavage of T13 was observed; with simple calculations a typical thrombin generation curve is obtained. Additions of rivaroxaban (5–50 μ g mL⁻¹) or apixaban (25–1000 μ g mL⁻¹) delayed the time to peak, and reduced the peak height and the area-under-the-curve, with higher doses blocking thrombin generation. Addition of dabigatran (25–400 μ g mL⁻¹) reduced peak height and area-under-the-curve; higher doses blocked thrombin generation.

Conclusion: We have developed a novel thrombin substrate that can monitor thrombin generation in whole blood without a2M correction. This allows for point-of-care determination of the presence of anticoagulants.

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OR303

Factor XIa and tissue factor activity in trauma patientsButenas S¹, Gissel M¹ and Park MS²¹Department of Biochemistry, University of Vermont, Colchester;²Division of Trauma, Critical Care, and General Surgery, Mayo Clinic, Rochester, USA

Background: It has been observed that trauma patients have an elevated procoagulant activity in their plasma. To some extent, this increased activity could be assigned to an elevated concentration of tissue factor (TF). However, in many instances there is a discrepancy between the levels of TF measured in trauma patient plasma and procoagulant activity observed. We hypothesized that factor (F)XIa could be responsible for an additional procoagulant activity.

Aims: To evaluate several groups of trauma patients for the presence of FXIa and TF in their plasma and to establish relationships between FXIa and TF and trauma severity.

Methods: Citrate plasmas from 98 trauma patients (47 blunt, 17 penetrating and 34 thermal) were evaluated in a clotting assay for the con-

centration of FXIa and TF using inhibitory antibodies against FXIa and TF.

Results: At admission (day 0), 23 patients (23%) had active TF and 46 (47%) and active FXIa in their plasmas. 19 of 23 patients (83%) with TF activity had active FXIa as well. Some patients without FXIa or TF activity at day 0 developed those activities in following days (days 1–5). A 'delayed' occurrence of TF activity was observed in 18 patients (18%) and that of FXIa in 17 (17%) trauma patients. There was direct correlation between the severity of trauma and FXIa occurrence. 35 of 57 (61%) patients with the injury severity score (ISS) > 25 and only 11 of 41 (27%) with the ISS $\leq$ 25 had active FXIa in their plasmas.

Conclusion: The majority of trauma patients have detectable FXIa activity in their plasma, with a significant fraction of them having TF activity as well. The frequency of FXIa correlates with the severity of trauma.

Disclosure of Interest: None declared.

OR304

DRVVT as simple method for monitoring patients with FXa inhibitors

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Background: Prothrombin time and aPTT are not suitable to monitor fXa inhibitors like rivaroxaban or apixaban. Different reagents give different results and the sensitivity is, especially at low inhibitor levels, not sufficient. The determination of anti Xa levels is not available in all labs, while thrombin generation measurements (TG) are expensive and time consuming. On the other site, DRVVT (Diluted Russell Viper Venom Time), known from Lupus anticoagulant testing, measures the conversion from fX to fXa and shows low inter-individually differences and high sensitivity to Xa inhibition.

Aims: Aim is the description of the relationship between fXa inhibitors and DRVVT.

Methods: The relationship between fXa inhibitors and DRVVT was investigated in *ex vivo* mixing experiments as well as patients ($N = 500$) with fXa inhibitor therapy. DRVVT was measured in comparison to PT, aPTT and anti Xa measurements. Additionally we calculated the thrombin concentration based on clot wave form analysis (CWFA) from aPTT and DRVVT measurements.

Results: There is a linear relation between DRVVT and rivaroxaban levels. DRVVT is more sensitive than TG and global routine testing especially in low ranges. Even at levels lower than 20 ng mL⁻¹ the DRVVT is slightly prolonged, whereas all the other tests are negative. We observe this constellation at 24–30 h after rivaroxaban application. The sensitivity of DRVVT is higher for rivaroxaban than for apixaban. *Ex vivo* and *in vivo* results are in agreement. Thrombin concentrations calculated from CWFA can be used for direct comparison of most anticoagulants.

Conclusion: DRVVT measurements are more sensitive than other coagulation methods to exclude fXa inhibitors; is cost effective and can be done to exclude rivaroxaban also in emergency units. The reagent is very stable and has a high robustness. Further investigations are necessary to investigate the differences in the sensitivity between apixaban and rivaroxaban.

Disclosure of Interest: None declared.

OR305

Efficacy and mechanism of clot contraction are determined by blood composition

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Background: The contraction of blood clots remains one of the least understood aspects of hemostasis and thrombosis, despite its potential clinical importance.

Aims: To investigate the kinetics of clot contraction and the mechanistic interplay between platelets, red blood cells (RBCs), fibrin(ogen), factor XIIIa, and thrombin.

Methods: A novel automated optical analyzer was used to follow the size of contracting clots.

Results: The rate and degree of clot contraction were critically affected by variation of platelet count, RBC volume fraction, fibrinogen concentration and fibrin crosslinking, thrombin activity, and platelet-fibrin interactions. Increasing platelet count above $250 \times 10^9 \text{ L}^{-1}$ revealed a switch from monophasic to biphasic contraction kinetics, reflecting complex platelet contractile machinery. Modifying the fibrin structure through fibrinogen levels revealed that denser fibrin was more resistant to contraction as the ability of the network to propagate the platelet contractile force was impaired. Inhibition of Factor XIIIa-mediated crosslinking also made clots contract less. Decreasing thrombin concentration resulted in weaker contraction, which can be attributed to reduced myosin phosphorylation and cytoskeleton remodeling within platelets. Comparison of clot contraction in DVT patients untreated and treated with warfarin revealed that, in addition to thrombin added, endogenous thrombin generation is required for clots to contract in a normal manner. Lastly, increasing the volume fraction of RBCs resulted in changes in the initial phase of contraction and the final degree of contraction due to the deformable mechanical nature of RBCs and their influence on the overall force balance of the contracting system.

Conclusion: Collectively, these results reveal the complementary and antagonistic roles of varying blood components in the multifactorial mechanism of clot contraction, as well as providing a basis for understanding of the clinical significance of contraction.

Disclosure of Interest: None declared.

Heparin and Heparinoids

OR306

In vivo characterization of anti-thrombotic and anti-inflammatory properties of new synthetic, protamine-reversible low molecular weight heparin

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Background: Low molecular weight heparins (LMWHs) are widely used to treat thrombotic disorders however clearance by kidney and lack of full reversibility limit treatment options. Previously we developed an innovative chemoenzymatic method to synthesize a new synthetic polysaccharide (dodecasaccharide; 12mer) with high anti-factor Xa (FXa) activity fully reversible with protamine.

Aims: To characterize the pharmacokinetic, anti-thrombotic and anti-inflammatory properties of 12mer *in vivo*.

Methods: Pharmacokinetic of 12mer was determined in time course (0.5–8 h, $n = 4$) and dose response (0.5–2 mg kg⁻¹, $n = 4$) studies using C57Bl/6J mice. Effect of 12mer (0.6 mg kg⁻¹), unfractionated heparin (UFH 150 U mg⁻¹), and enoxaparin (1 mg kg⁻¹) on fibrin accumulation was analyzed in femoral vein thrombosis model induced by electrolytic injury. We also investigated if 12mer inhibits thrombotic and inflammatory effect mediated by FXa in mouse model of sickle cell disease. Sickle mice received s.c. injection of saline ($n = 6$) or 12-mer ($n = 6$; 2 mg kg⁻¹) every 8 h for 7 days.

Results: The half-life of the 12mer (1.5 mg kg⁻¹; s.c.) was 3 h and 12mer was cleared from the circulation by 8 h. Fibrin accumulation was similarly reduced by 12mer (1.63 ± 0.48), UFH (1.26 ± 0.35) and enoxaparin (1.39 ± 0.85 , $P < 0.05$ for all) compared to vehicle treated mice (2.99 ± 1.16 ; $P < 0.05$; relative fluorescent intensity mean $\pm$ SD). Protamine reversed the anti-thrombotic effect of 12mer and returned fibrin accumulation to normal levels (2.69 ± 0.87 ; $P < 0.05$ vs. 12mer alone).

Finally, compared to saline, 12mer reduced plasma levels of thrombin anti-thrombin complexes (8.2 ± 1.4 vs. $4.4 \pm 1.3 \text{ ng mL}^{-1}$; $P = 0.07$, mean $\pm$ SEM), IL-6 (5.5 ± 1.5 vs. $1.1 \pm 0.4 \text{ pg mL}^{-1}$, $P < 0.05$) and sVCAM1 (1.1 ± 0.2 vs. $0.6 \pm 0.07 \text{ } \mu\text{g mL}^{-1}$, $P < 0.05$) in sickle mice and blocked histone mediated ECs injury *in vitro*.

Conclusion: Our data indicates that chemoenzymatic synthesis offers a promising approach to obtain new, protamine-reversible LMWHs with high anti-coagulant and anti-inflammatory properties.

Disclosure of Interest: None declared.

OR307

Partially desulfated heparin modulates the interactions between anti-protamine/heparin antibodies and platelets

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Background: Protamine (PRT) is the standard drug to neutralize heparin. PRT/heparin complexes induce an immune response similar to that observed in heparin-induced thrombocytopenia (HIT). Partially desulfated heparin (ODSH) was shown to interfere with anti-platelet factor 4/heparin antibodies (Abs), which are responsible for HIT.

Aims: To analyze the impact of ODSH on the interactions between anti-PRT/heparin IgG Abs and platelets (PLTs).

Methods: The impact of ODSH on anti-PRT/heparin Ab-induced PLT-activation was assessed using modified heparin-induced PLT aggregation (mHIPA) test. The ability of ODSH to prevent anti-PRT/heparin Ab-induced PLT-destruction *in vivo* was investigated using the NOD/SCID mouse model. The interaction between PRT/heparin-complexes and PLTs in the presence of ODSH was analyzed using flow cytometry (FC).

Results: In mHIPA, PLT-activation by anti-PRT/heparin Abs was largely inhibited at a concentration higher than $16 \text{ } \mu\text{g mL}^{-1}$ ($P < 0.001$). *In vivo* studies showed that ODSH improved PLT-survival in the presence of PRT, heparin and anti-PRT/heparin Abs (median PLT-survival with $20 \text{ } \mu\text{g mL}^{-1}$ ODSH: 75%, range 70–81 vs. without ODSH: 49%, range 44–59, $P = 0.006$). Interestingly, even when ODSH was administered into the mice 1 h after Abs injection PLT-survival was higher compared to buffer (median PLT-survival with $20 \text{ } \mu\text{g mL}^{-1}$ ODSH: 83%, range 77–93% vs. without ODSH: 59%, range 29–61%, $p = 0.02$). FC studies showed that ODSH not only inhibits complex binding to PLTs (mean fluorescence intensity [MFI] without ODSH: 85 ± 14 vs. $4 \text{ } \mu\text{g mL}^{-1}$ ODSH: 15 ± 0.6 , $P = 0.013$) but also displaces pre-bound complexes from PLT surface (complex binding: without ODSH: 324 ± 43 vs. $32 \text{ } \mu\text{g mL}^{-1}$ ODSH: 53 ± 9 , $P = 0.0007$).

Conclusion: Our studies demonstrate that ODSH is able to prevent anti-PRT/heparin IgG Ab-mediated PLT-activation and -destruction suggesting a therapeutic benefit of combining ODSH with heparin to reduce anti-PRT/heparin Ab-mediated complications.

Disclosure of Interest: None declared.

OR308

Thermal stability of platelet factor 4 and its complexes with heparins studied by differential scanning calorimetry

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Background: Heparins are sulfated glycans clinically used to treat thrombotic disorders. They bind to endogenous protein platelet factor 4 (PF4) forming PF4/heparin complexes which may cause immune-mediated adverse drug reactions (e.g. heparin-induced thrombocytopenia). This adverse drug reaction is likely a misdirected bacterial host defense mechanism with platelets covered with heparin and PF4 mimicking PF4-coated bacteria. Recently, we have shown that heparins induce changes in PF4, resulting in PF4/heparin antibody binding (Blood 2014).

Aims: We characterize here the thermal stability of PF4 and its complexes with unfractionated heparin and heparins of defined chain length (5-, 6-, 8-, and 16-mers).

Methods: Differential scanning calorimetry was used to investigate the thermal stability of PF4 and PF4/heparin complexes by heating the protein and protein/heparin complexes and measuring the temperature dependent heat capacity of the solution. The antigenicity of PF4/heparin complexes was assessed by enzyme immunoassay with sera of patients known to contain anti-PF4/heparin antibodies.

Results: We found that PF4 behaves as a heat-stable protein unfolding at a melting temperature (T_m) of 96 °C. Interestingly, T_m rises with increasing PF4 concentration, indicating that PF4 unfolds in tetrameric form. A similar rise in T_m is induced when PF4 complexes with heparin. Heparins stabilize PF4 against unfolding, resulting in increasing melting temperatures of PF4/heparin complexes. The complexes formed by PF4 with longer heparins are antigenic (bind anti-PF4/heparin antibodies) and are more stable than those formed with non-antigenic shorter heparins. However, although very high heparin concentrations reduce PF4/heparin antibody binding, they do not result in a decrease of the melting temperature.

Conclusion: Thermal stability of PF4/heparin complexes is associated with their antigenicity. Our observations are important for designing new non-antigenic heparin-derived drugs.

Disclosure of Interest: None declared.

OR309

Comparison of bleeding complications between Anti-Xa and aPTT monitoring in patients receiving unfractionated heparin

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Background: Antifactor Xa monitoring (Anti-Xa) has been shown to be a more precise monitoring tool than activated partial thromboplastin (aPTT) for patients administered unfractionated heparin (UFH) anticoagulation therapy.

Aims: The study aim was to compare bleeding complications (BC) between Anti-Xa and aPTT monitored UFH patients.

Methods: A retrospective cross-sectional study was conducted on patients diagnosed with acute coronary syndrome (ACS, $N = 14,822$), ischemic stroke (STK, $N = 1568$) or with primary diagnosis of venous thromboembolism (VTE, $N = 4414$) in the MedAssets data from January 2009 to December 2013. Anti-Xa and aPTT cohorts were identified from hospital billing detail using both brand and generic name as search criteria. Propensity score techniques were used to match Anti-Xa cases to aPTT controls. BCs were evaluated using protamine sulfate (PS) administration and red blood cell transfusions (RBCT) identified from hospital billing data. Multivariable logistic regression was used to identify significant drivers of BCs.

Results: Anti-Xa patients had fewer RBCTs than aPTT patients in ACS (7.0% vs. 24.6%, $P < .0001$), STK (13.8% vs. 21.9%, $P < .0001$), and VTE (3.9% vs. 8.6%, $P < .0001$). After controlling for patient age and gender, diagnostic risks (e.g. anemia, renal insufficiency, trauma) and invasive procedures (e.g. cardiac catheterization, hemodialysis, coronary artery bypass graft) Anti-Xa patients were less likely have a RBCT in ACS (OR = 0.16, CI = 0.14–0.18, $P < .0001$), STK (OR = 0.41, CI = 0.29–0.57, $P < .0001$), and VTE (OR = 0.35, CI = 0.26–0.48, $P < .0001$). No differences were seen in PS use between Anti-Xa and aPTT patients in STK or VTE. In ACS, Anti-Xa patients had fewer PS administrations (15.5% vs. 19.8%, $P < .0001$) even after controlling for patient age and gender, diagnostic risks and invasive procedures (OR = 0.60, CI = 0.52–0.70, $P < .0001$).

Conclusion: Anti-Xa was associated with fewer complications of bleeding as manifested by a significant reduction in RBCTs when compared to patients monitored by aPTT alone.

Disclosure of Interest: K. Belk Grant/Research Support from: Instrumentation Laboratory, M. Laposata Consultant for: Instrumentation Laboratory, C. Craver Grant/Research Support from: Instrumentation Laboratory.

OR310

Prophylactic enoxaparin in critically ill patients with severe renal insufficiency

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Background: In patients with severe renal insufficiency, enoxaparin 30 mg daily is the recommended dosing regimen to avoid potential bio-accumulation. To date, no study has evaluated the safety of using enoxaparin 30 mg daily in critically ill patients with severe renal insufficiency.

Aims: To determine whether the use of enoxaparin prophylaxis at 30 mg daily in critically ill patients with severe renal insufficiency (creatinine clearance (CrCl) ≤ 30 mL min⁻¹) is associated with clinically significant bio-accumulation.

Methods: In this prospective single-center cohort study, we included patients > 18 years who were expected to be admitted to intensive care (ICU) for ≥ 72 h with a CrCl of ≤ 30 mL min⁻¹. The primary outcome was bio-accumulation defined as a trough anti-Xa level above 0.40 IU mL⁻¹. Trough anti-Xa levels were measured on days 1–4, 7, and 10. Secondary outcome measures included major bleeding episodes and venous thromboembolic events.

Results: Twenty patients with median age of 62 years (range 41–86) and median APACHE II score of 30 (range 17–45) were enrolled. The median CrCl was 25 mL min⁻¹ (range 8–30 mL min⁻¹) at enrolment. Fifteen patients received dialysis during ICU stay. Trough anti-Xa levels were measured on 75 occasions. No bio-accumulation of enoxaparin was detected and 95% of anti-Xa levels were undetectable (< 0.10 IU mL⁻¹). One patient received blood transfusion on study day 10 for progressive anaemia associated with rectal bleeding and

bleeding at dialysis catheter site; the trough anti-Xa level was 0.26 IU mL^{-1} . There were no thrombotic events.

Conclusion: Our study suggests that a thromboprophylactic dose of enoxaparin 30 mg daily in critically ill patients with severe renal insufficiency does not seem to bio-accumulate. The clinical efficacy and safety profile remains to be assessed.

Disclosure of Interest: None declared.

Microparticles – II

OR311

Leucocytes MPs promote clot dissolution in a uPA/uPAR dependant manner

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Background: Microparticles (MPs) are small vesicles well known for their procoagulant properties. We recently reported a profibrinolytic function of MPs supported by circulating leukocytes derived MPs (Leu-MPs), able to generate plasmin (MP-PGC) via the urokinase (uPA) system.

Aims: Therefore, we hypothesized that Leu-MPs may promote clot dissolution. To that aim, using fibrinolysis models, we investigated the lytic capability of MPs derived from myeloid cells, *in vitro* and *ex vivo*.

Methods: MPs generated in inflammatory conditions were purified from 1/linear myeloid human cells (myel-MPs), 2/bacterial endotoxin-stimulated blood (tox-MPs), 3/plasma from sepsis patients (seps-MPs) with three levels of MP-PGC. Their lytic effect on a clot formed *in vitro* was investigated using 1/a fluorescent clot lysis test and 2/a lysis front retraction assay by video fluorescent microscopy.

Results: Compared to a negative control, we observed that myel- and tox-MPs induce a significant dose- and time-dependent increase of fluorescent release from clot ($2100 \pm 1600 \text{ a.u.}$ vs. $520 \pm 120 \text{ a.u.}$ at $5.10^6 \text{ MPs per 24 h}$, respectively, $P < 0.05$). These MPs also promote a significant MP-dependent retraction of lysis front ($63 \pm 42\%$ vs. $9 \pm 4\%$ lysed area, at $10^6 \text{ MPs per 48 h}$, $P < 0.05$). Interestingly, seps-MPs also promote clot dissolution according to the level of MP-PGC. This effect was mediated by Leu-MPs, as demonstrated by specific MPs subpopulations depletion experiments. Moreover, this lytic activity was significantly decreased in presence of inhibitors such as $\alpha 2$ -antiplasmin or an anti-uPA antibody. Interestingly, the lytic effect was amplified when uPA receptors on the MP surface were saturated by exogenous uPA ($1750 \pm 600 \mu\text{m}^2 \text{ min}^{-1}$ vs. $930 \pm 300 \mu\text{m}^2 \text{ min}^{-1}$, at 48 h, $P < 0.05$).

Conclusion: Altogether, this work demonstrated that Leu-MPs promote clot dissolution in a uPA/uPAR-dependent manner. Although these results need to be confirmed *in vivo*, this MP-dependent fibrinolytic activity may counterbalance their well- described procoagulant activity.

Disclosure of Interest: None declared.

OR312

The change of the amount of circulating microparticles and their association to the general atherosclerotic burden after acute coronary syndrome

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Background: Circulating microparticles (MPs) are formed upon activation and might be used as biomarkers for risk stratification in coronary

artery disease but little is known of the change of the MPs concentrations after an acute coronary syndrome (ACS).

Aims: To evaluate the change of MPs up to 2 years after ACS.

Methods: One hundred and five patients with ACS were included in an observational platelet study and followed for 2 years. MPs were evaluated in whole blood by flow cytometry at inclusion, after 1–2 weeks, 3 months, 1 and 2 years. The general atherosclerotic burden was examined by coronary angiogram, ultrasound of the carotid arteries and ankle-brachial index for determination of peripheral atherosclerosis.

Results: The mean age was 68 ± 10 years in the ACS group and 48% had a non-ST-elevation myocardial infarction as the index event. 97% and 95% were treated with aspirin and P2Y₁₂-receptor blocking agent, respectively. The type of ACS was not related to the concentrations of platelet, endothelial or monocyte derived MPs. The majority of the circulating MPs, 2894 (2262–3950)/10,000 platelets, expressed CD41 and their concentration did not change during the first year after the ACS. The concentrations of CD62P+, CD144 + and CD14 + MPs were low and did not change significantly during the first year. 2 years after the ACS the levels of CD41 + and CD62P+MPs were increased compared to the other time-points ($P < 0.0001$). Patients with manifest atherosclerosis in the coronary, carotid and peripheral arteries had higher concentrations of platelet derived MPs ($P = 0.01$ – 0.03) despite treatment with double platelet inhibition after the ACS.

Conclusion: In an ACS population the majority of MPs are platelet derived and there is no dynamic change of concentrations during the first year after the acute event. Patients with an increased general atherosclerotic burden have higher amounts of circulating platelet derived MPs and further research regarding optimal antithrombotic treatment in this group of patients is warranted.

Disclosure of Interest: None declared.

OR313

Decreased microparticle-associated tissue factor activity in morbid obese patients after bariatric surgery

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Background: Tissue factor (TF) is the main *in vivo* initiator of the blood coagulation cascade. Active circulating TF was detected on small negatively charged membrane vesicles, so called microparticles (MPs), which are released upon cell activation and apoptosis from a variety of cells. Increased coagulation activation was found in morbid obese patients and elevated levels of TF-bearing MPs may contribute to the prothrombotic state in these patients.

Aims: To determine MP-associated TF activity levels in morbid obese patients before and after weight loss due to bariatric surgery.

Methods: MP-TF activity was measured with a factor Xa generation assay in morbid obese patients before and 2 years after bariatric surgery. In addition, clinical parameters were determined.

Results: Seventy-four morbid obese patients (mean age: 42 (± 11) years; 61 female) were included in this study. After bariatric surgery the body mass index decreased from (mean $\pm$ SD) 47 ± 6 to $32 \pm 7 \text{ kg m}^{-2}$ ($P < 0.001$) and a significant improvement of metabolic parameters was observed. Postoperatively the mean MP-TF activity decreased significantly from $0.32 \pm 0.43 \text{ pg mL}^{-1}$ to $0.18 \pm 0.41 \text{ pg mL}^{-1}$ ($P = 0.042$). Preoperative MP-TF activity correlated with CRP levels ($r = 0.3$; $P = 0.02$).

Conclusion: We could demonstrate a significant decrease of MP-TF activity after weight loss in morbid obese patients. Decreased MP-TF activity might contribute to an improved coagulation profile of morbid obese patients after weight loss.

Disclosure of Interest: None declared.

OR314

Proteolytic activity of circulating microparticles from ischemic stroke patients revealed by single particle laser scanning confocal microscopy

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Background: Cell-derived microparticles (MPs) are released as a response to cell-activation in all body fluids. Recently, the presence of proteases at the MPs surface has been described in several diseases. Given the suspected roles of these MPs in vascular biology, their characterization in patients with thrombosis-related disorders may be of diagnostic and prognostic values.

Aims: Since classical methods of MPs detection are unable to measure the proteolytic activity of protease-bearing MPs, we developed a laser-scanning confocal microscopy (LSCM) method that allows *in situ* detection and quantification of the individual MPs proteolytic potential. Then, in order to investigate its feasibility in pathological conditions, we studied fibrinolytic-MPs in ischemic stroke patients during the acute phase: before, during and after tissue-type plasminogen activator (tPA) infusion.

Methods: MPs were purified from different cultured cell-lines and plasma samples by sequential ultra-centrifugation, as previously reported and then, fluorescently labelled, surface-immobilised and detected using high-resolution LSCM.

Results: There was a linear relationship between surface-immobilized MPs number, concentration and size, confirming the feasibility to quantify MPs in fluids over a wide concentration. Then, we detected tPA and plasmin activity (by specific fluorogenic substrates) at the surface of purified MPs from different cultured cell-lines overexpressing tPA. Lastly, we purified MPs from plasma samples from a cohort of ischemic stroke patients. We demonstrated that i) a low concentration of tPA-bearing MPs is present in ischemic stroke patients during the acute phase, ii) this concentration transiently increases after tPA-induced thrombolysis and iii) tPA-bearing MPs display proteolytic activity and are able to generate active plasmin.

Conclusion: Therefore, this novel method unveils tPA-bearing MPs as potential fibrinolytic players during acute ischemic stroke and after thrombolysis.

Disclosure of Interest: None declared.

OR315

Detection of procoagulant and profibrinolytic epcam-positive microparticles in pleural fluid: a new approach for the diagnosis of the tumoral origin of pleural effusions

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Background: Non-invasive biomarkers for discriminating benign from malignant pleuritis are needed. Tumor cells produce microparticles.

Aims: Therefore, we hypothesized that tumor-derived microparticles could be present in the pleural liquid and could help to identify patients with malignant pleural effusions by a non-invasive method.

Methods: Fifty patients with pleural effusions were included in this study. Among them, 39 consecutive patients had a histologically confirmed tumor (adenocarcinoma (ADK), $n = 17$ or non-ADK neoplasia, $n = 22$) and 11 patients with a non-tumoral pleural effusion. Microparticles were enumerated by high sensitive flow cytometry and ELISA. MP-dependent tissue factor activity and the MP-dependent plasmin generation capacity of these MP was evaluated according to their cellular origin.

Results: Using highly sensitive flow cytometry and cryo-electron microscopy, this study showed the presence of large amount of microparticles in pleural effusions. Among them, we demonstrated the presence of tumor-derived microparticles expressing EpCAM (epithelial-cell-adhesion-molecule) in the pleural fluids from ADK patients. In our cohort, no benign pleuritis (0/11) contained EpCAM+ MPs. In contrast, EpCAM+ MPs were found in 11/39 malignant pleuritis. These EpCAM+MPs were both highly procoagulant and profibrinolytic. Interestingly 3 of the ADK patients were negative by cytology. Thus, the detection of procoagulant and profibrinolytic EpCAM-positive microparticles was shown to be useful as a tool to complement cytology for better diagnoses of malignant pleuritis.

Conclusion: To our knowledge, this is the first work to directly identify tumoral microparticles in pleural liquids using EpCAM associated with coagulolytic functional assays. This study establishes the basis for a potential biomarker for diagnosing and monitoring malignant pleuritis.

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Non-vitamin K oral anticoagulants and reversal

OR316

Thrombotic safety of a four-factor prothrombin complex concentrate (Beriplex P/N) for edoxaban reversal in a rabbit model

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Background: *In vivo* animal data have shown four-factor prothrombin complex concentrate (4F-PCC) to be effective in preventing bleeding induced by excessive plasma levels of the new oral anticoagulants (NOAC) including the target specific FXa inhibitor edoxaban.

Aims: This study was designed to determine the risk of thrombosis associated with administration of a 4F-PCC within the context of edoxaban reversal.

Methods: The thrombogenicity of a 4F-PCC (Beriplex® P/N) was assessed in female New Zealand White rabbits using a modified Wessler stasis model. Animals received the maximum clinical dose of 4F-PCC (50 IU kg⁻¹) or multiples thereof (300 IU kg⁻¹) followed by intravenous administration of the direct factor Xa inhibitor edoxaban (300, 600 µg kg⁻¹) into the contralateral ear vein. Administration of isotonic saline was used as negative control. FEIBA® NF 500 E (50 U kg⁻¹) served as the positive control. Shortly after infusion, venous stasis was induced in an isolated jugular vein segment over 30 min followed by determination of thrombosis score and wet weight.

Results: Following venous stasis, no clotting was observed after isotonic saline infusion or 50 IU kg⁻¹ 4F-PCC, whereas fully occlusive clots formed after FEIBA treatment. Non-occlusive clots developed after treatment with supratherapeutic 4F-PCC doses of 300 IU kg⁻¹. Co-administration of edoxaban (600 µg kg⁻¹) fully inhibited clot formation in all animals tested ($P < 0.01$).

Conclusion: This study indicates no thrombogenic signal after treatment with the 4F-PCC studied when dosed within the clinical dose range. The presence of edoxaban reduced the thrombogenicity of supra-therapeutic 4F-PCC doses confirming similar data previously generated for dabigatran co-administration. Therefore, the risk of thrombosis appears to be constrained both by residual NOAC levels present and the composition of the 4F-PCC evaluated.

Disclosure of Interest: E. Herzog Employee of: Employee of CSL Behring GmbH, F. Kaspereit Employee of: Employee of CSL Behring GmbH, W. Kregge Employee of: Employee of CSL Behring GmbH, B. Doerr Employee of: Employee of CSL Behring GmbH, Y. Morishima Employee of: Employee of Daiichi Sankyo, G. Dickneite Employee of: Employee of CSL Behring GmbH.

OR317

Reversing the effect of rivaroxaban – an *in-vitro* study

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Background: Specific antidotes to reverse the anticoagulant effect of non-Vit K antagonist oral anticoagulants (NOACs) are not available, and prothrombin complex concentrate (PCC), activated PCC (aPCC)

and recombinant factor VIIa (rVIIa) are variously used as surrogate in case of bleeding.

An increasing numbers of patients are treated with DOACs, but the optimal way to reverse their effect in case of bleeding is not known.

Aims: To detect the most effective haemostatic agent and appropriate dose for reversal of the anticoagulant effect of rivaroxaban.

Methods: Blood added with CTI from 29 patients on rivaroxaban were spiked with PCC, aPCC and rVIIa at concentrations imitating 80%, 100% and 125% of the recommended therapeutic doses (40 IU, 50 IU and 90 µg kg⁻¹). The reversal effect was assessed by thrombin generation assay in platelet poor plasma and thrombelastography in whole blood. Healthy subjects ($n = 33$) were used as controls.

Results: Clotting time in whole blood and lag time in plasma were significantly prolonged in rivaroxaban patients compared to controls (1142s vs. 739s and 2.9 min vs. 8.9 min). ETP and peak height in plasma were reduced (1344 nM min⁻¹ vs. 659 nM min⁻¹ and 157.4 nM vs. 24.2 nM). Other measured parameters were not affected. All tested haemostatic agents improved coagulation measurements at least to the level of the controls. The maximum effect was obtained by aPCC at 100% of the recommended dose where CT, LT, ETP and peak exceeded the control values. Reducing the aPCC dose to 80% diminished the difference in CT and ETP significantly, but it was still more effective than the other haemostatic agents. Increasing the dose to 125% did not improve the effect.

Conclusion: aPCC reversed the parameters affected by Rivaroxaban more effectively than PCC and rFVIIa. These data may suggest that an aPCC dose corresponding to 30–50 IU kg⁻¹ is the preferred treatment option in case of life-threatening bleeds.

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OR318

Andexanet alfa but not four-factor prothrombin complex concentrate reverses rivaroxaban-induced anticoagulation as measured by reduction in blood loss in a rabbit liver laceration model

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Background: Andexanet alfa (AnXa) is a modified recombinant Factor Xa (fXa) derivative that reverses fXa inhibitor-mediated anticoagulation in animals and humans by binding direct fXa inhibitors. AnXa reduces anti-fXa activity and the plasma free fraction of fXa inhibitors, restoring hemostasis as evidenced by normal thrombin generation and correction of PT and aPTT (Lu et al., Nat. Med 2013). As the use of direct fXa inhibitors has increased, so has the need for an antidote to reverse bleeding as none is currently approved.

Aims: The objective of these studies was to compare the activity of Kcentra®, a 4-factor prothrombin complex concentrate (PCC) to AnXa in reversing rivaroxaban (riva) anticoagulation in a rabbit model of bleeding, as assessed by coagulation markers and blood loss (BL). While Kcentra has been approved by the FDA for use in warfarin-treated patients with over-anticoagulation or bleeding, the potential use of PCCs in the reversal of bleeding in fXa inhibitor-treated patients is less clear and likely pro-coagulant.

Methods: NZW rabbits were treated with riva (IV, 1 mg kg⁻¹) and 30 min later, either PCC (25 or 50 IU kg⁻¹) or AnXa (75 mg per rabbit) was administered IV. Liver injury was then induced with 10 1-cm incisions following laparotomy, and BL was measured for 15 min.

Plasma levels of AnXa, riva, and anti-fXa activity, and PT, and aPTT were determined.

Results: AnXa reduced BL by > 80% in riva-anticoagulated rabbits, similar to what was observed in previous studies. Significant reversal of BL, anti-fXa activity, PT and aPTT was observed in rabbits treated with AnXa ($P < 0.05$ vs. riva), and riva was redistributed from the extravascular to the intravascular compartment. Therapeutic doses of PCCs had no effect on any of these markers in riva-anticoagulated rabbits ($P > 0.05$ vs. riva).

Conclusion: Andexanet alfa was shown to be a highly effective antidote to address bleeding due to riva anticoagulation. In contrast, Kcentra showed no reversal activity in this model as assessed by BL or pharmacodynamics markers.

Disclosure of Interest: P. Pine Shareholder of: Portola Pharmaceuticals, Inc., Employee of: Portola Pharmaceuticals, Inc., S. Hollenbach: None declared, F. DeGuzman Employee of: Portola Pharmaceuticals, Inc., S. Tan Employee of: Portola Pharmaceuticals, Inc., J. Malinowsky Employee of: Portola Pharmaceuticals, Inc., P. Pratikhya Employee of: Portola Pharmaceuticals, Inc., G. Lu Shareholder of: Portola Pharmaceuticals, Inc., Employee of: Portola Pharmaceuticals, Inc., J. Curnutte Shareholder of: Portola Pharmaceuticals, Inc., Employee of: Portola Pharmaceuticals, Inc., P. Conley Shareholder of: Portola Pharmaceuticals, Inc., Employee of: Portola Pharmaceuticals, Inc.

OR319

Reversal of targeted anticoagulants by complexes of alpha-2-macroglobulin with thrombin and factor Xa

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Background: Drugs to reduce coagulation function are a mainstay of antithrombotic therapy. Recently developed therapies have used oral anticoagulants that target the active sites of thrombin (FIIa) or factor Xa (FXa). Existing coagulation tests are being adapted to measure plasma levels of these drugs but have not settled on a defined standard test.

Aims: The aim of these studies is to test the ability of complexes of FIIa and FXa with alpha-2-macroglobulin to bind to these targeted anticoagulants.

Methods: Rivaroxaban, which targets FXa, was isolated from tablets by ethanol extraction. The active form of dabigatran (BIBR 953), which targets FIIa, was purchased from Selleck (Houston, TX). Alpha-2-macroglobulin (a2M) was purified from plasma; FIIa and FXa were also plasma derived. a2M complexes with FIIa (a2M-IIa) and FXa (a2M-Xa) were made by incubating the enzymes with a2M. The complex was purified away from uncomplexed molecules by ion exchange chromatography. Bleeding was assessed in mice by injury to the saphenous vein.

Results: a2M-IIa and a2M-Xa cleaved their respective synthetic substrates. a2M-IIa cleavage of synthetic substrate was blocked by dabigatran, but not by rivaroxaban or antithrombin/heparin. a2M-Xa cleavage of synthetic substrate was blocked by rivaroxaban, but not by dabigatran, TFPI, or antithrombin/heparin (LMWH). Dabigatran binding to a2M-IIa and rivaroxaban binding to a2M-Xa, as measured by competition with substrate cleavage, was dose dependent with a dissociation constant similar to that observed for the uncomplexed enzyme. Bleeding was induced in mice by administration of dabigatran or rivaroxaban. a2M-IIa corrected bleeding induced by dabigatran and a2M-Xa corrected bleeding induced by rivaroxaban.

Conclusion: Complexes of thrombin and factor Xa with alpha-2-macroglobulin can assay for the presence and level of targeted oral anticoagulant agents, even in the presence of heparin. These complexes can reverse bleeding associated with the targeted anticoagulants.

Disclosure of Interest: None declared.

OR320

Idarucizumab does not have prothrombotic effects: assessment of coagulation markers in healthy volunteers

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Background: Idarucizumab, a specific dabigatran reversal agent, has shown immediate, complete, and sustained reversal of dabigatran-induced anticoagulation in healthy volunteers including in the elderly and in those with renal impairment.

Aims: There is evidence that certain reversal agents for anticoagulants can exhibit prothrombotic effects during use, which may lead to complications. An optimal reversal agent should therefore not exhibit this effect. For this reason we assessed the effect of idarucizumab on the following biomarkers, indicative for a possible prothrombotic state: thrombin generation (TG), D-dimer, and prothrombin fragment 1.2 (F1.2) levels. Samples from two phase I, placebo-controlled clinical trials in Caucasian and Japanese subjects were used.

Methods: The effect of 4 g and 8 g of idarucizumab or placebo on TG in plasma was measured by calibrated automated thrombogram before and after the infusion in 8 volunteers per dose group. TG over time was characterized by lag time, time to peak, peak height and area under the curve (AUC). Levels of D-dimer and F1.2 were assessed up to 72 h after the end of infusion of 1–8 g idarucizumab using validated ELISA methods. Ethics committee approval and subjects' informed consent were obtained.

Results: There were no differences in TG (lag time, time to peak, peak height and AUC) vs. baseline and placebo, at 15 min, 4 h and 8 h after the end of idarucizumab infusion. In both the Japanese and the Caucasian group, D-dimer and F1.2 levels measured over 72 h after infusion of up to 8 g idarucizumab did not change significantly compared to placebo. Levels in samples from the Caucasian study were more variable than in the Japanese study.

Conclusion: Administration of idarucizumab to healthy volunteers did not show any effect on prothrombotic biomarkers, suggesting that idarucizumab does not have a prothrombotic effect. RE-VERSE AD, an ongoing clinical trial, may provide the clinical data to support this finding.

Disclosure of Interest: J. Stangier Employee of: Boehringer Ingelheim, M. Schmohl Employee of: Boehringer Ingelheim, S. Glund Employee of: Boehringer Ingelheim, A. Harada Employee of: Boehringer Ingelheim, S. Imazu Employee of: Boehringer Ingelheim, M. De Smet Employee of: Boehringer Ingelheim, V. Moschetti Employee of: Boehringer Ingelheim, S. Ramael Employee of: SGS Life Science Services, I. Ikushima Employee of: Medical Co. LTA Sumida Hospital, F. Gruenenfelder Employee of: Boehringer Ingelheim, P. Reilly Employee of: Boehringer Ingelheim.

Venous thromboembolism epidemiology III

OR321

Prognostic significance of asymptomatic deep vein thrombosis in patients presenting with acute symptomatic pulmonary embolism

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Background: Concomitant deep vein thrombosis (DVT) in patients with acute pulmonary embolism (PE) has a higher short-term risk for all-cause death, PE-related death, and recurrent VTE than patients solely diagnosed with PE.

Aims: To assess whether PE patients with concomitant asymptomatic DVT have an increased risk of short-term complications.

Methods: We prospectively examined 820 normotensive patients with PE enrolled in the multicenter PROTECT study. Patients underwent bilateral lower extremity venous compression ultrasonography (CCUS) to assess for concomitant DVT. The primary study outcome, all-cause mortality, and the secondary outcome of PE-specific mortality were assessed during the first month of follow-up after PE diagnosis. Multivariate logistic regression adjusted for significant covariates.

Results: Of 820 patients diagnosed with PE, 46% (375/820) had concomitant DVT, and signs or symptoms of DVT were absent in 51% (193 of 375 patients) of these patients. Overall, 37 out of 820 patients died (4.5%; 95% confidence interval [CI], 3.1% to 5.9%). Eleven patients (11 of 820 patients; 1.3%; 95% CI, 0.6% to 2.1%) died from PE, and 26 (26 of 820 patients; 3.2%; 95% CI, 2.0% to 4.4%) died from other causes. Other causes of death consisted of cancer (1.2%; 10 of 820 patients), congestive heart failure (0.5%; 4 of 820 patients), respiratory failure (0.4%; 3 of 820 patients), major bleeding (0.4%; 3 of 820 patients), and miscellaneous diseases (0.7%; 6 of 820 patients). Patients with concomitant asymptomatic DVT had an increased all-cause mortality (odds ratio [OR] 2.77; 95% CI, 1.35–5.67; $P = 0.005$) and PE-specific mortality (OR 7.11; 95% CI, 1.42–35.53; $P = 0.02$). No variables showed evidence of confounding for the effect of asymptomatic DVT on the outcome undergoing analysis.

Conclusion: In stable patients with an episode of acute symptomatic PE, the presence of concomitant asymptomatic DVT is an independent predictor of death in the ensuing month after diagnosis.

Disclosure of Interest: None declared.

OR322

The increased risk of arterial cardiovascular disease in patients with prior venous thrombosis is not explained through elevated levels of procoagulant factors

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Background: Patients with venous thrombosis (VT) are at increased risk of subsequent arterial cardiovascular disease (CVD) which is at least in part explained by common etiological risk factors.

Aims: We aimed to explore whether common etiological risk factors are associated with levels of procoagulant factors (factor VIII[FVIII], von Willebrand factor [vWF] and fibrinogen), and whether the relationship between these risk factors and subsequent CVD could be explained through procoagulant factors in patients with VT.

Methods: We used data from the MEGA follow up study in which patients with VT were followed for an average of 5.5 years, completed detailed questionnaires on risk factors for VT and CVD and had blood taken. Linear and Cox regression analyses were used to analyze the relations between risk factors (malignancy, raised BMI, smoking, chronic illnesses, dyslipidemia, impaired kidney function and diabetes), procoagulant factor levels and risk of CVD.

Results: In 2178 VT patients, 50 CVD events occurred for an incidence rate of 3.8 per 1000 person years (95% CI, 2.8–5.0). The presence of all tested risk factors, except for smoking, was associated with elevated levels of FVIII, vWF and fibrinogen. There was a stepwise increase in risk of CVD with increasing procoagulant factor levels, with the highest hazard ratios in the > 97.5th percentile: 2.2 (95% CI: 0.8–5.6) for FVIII, 5.8 (95% CI: 1.3–25.3) for vWF and 11.8 (95% CI: 3.6–39.3) for fibrinogen compared with patients with levels of these factors at the ≤ 25th percentile. Additionally, every common risk factor was (as expected) associated with an increased risk of CVD but adjustments for procoagulant factors in a mediation analysis did not attenuate the aforementioned relationship.

Conclusion: In patients with previous VT, common risk factors were associated with increased procoagulant factor levels. However, these were not causally related with CVD but may be markers of resultant endothelial damage and inflammation.

Disclosure of Interest: None declared.

OR323

Incidence of venous thromboembolism in western france: comparison between 1998 and 2013

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Background: In 1998, in a well-defined geographic area of Western France, we estimated the overall annual incidence of venous thromboembolism (VTE) [Oger E. *Thromb Haemost* 2000]. Since then, population characteristics and VTE management have changed.

Aims: To estimate the incidence of VTE in 2013, and to compare it to that of 1998.

Methods: Using the same diagnostic criteria in the same geographic area than in 1998, we prospectively recorded all cases of pulmonary embolism (PE) and deep vein thrombosis (DVT) of the lower limbs diagnosed between March 1st, 2013 and February 28th, 2014 in hospitals and in the community. The 2013 incidence rates of VTE were compared with those of 1998 using age- and sex-specific standardized incidence ratios (SIRs).

Results: In 2013, we recorded 576 VTE cases (279 isolated DVT and 297 PE ± DVT). Among 367,911 inhabitants, the incidence of VTE was 1.57 per 1000 (95% Confidence Interval (CI), 1.44–1.69); the incidence of isolated DVT was 0.76 per 1000 (95% CI, 0.67–0.85) and that of PE (±DVT) was 0.81 per 1000 (95% CI, 0.72–0.90). In 1998, incidences per 1000 inhabitants were 1.83 (95% CI, 1.69–1.98) for VTE, 1.24 (95% CI, 1.12–1.36) for DVT and 0.60 (95% CI, 0.52–0.69) for PE. Standardization showed that the overall VTE incidence was significantly lower in 2013 as compared with 1998 (SIR 0.72 (95% CI 0.67–0.79)) as well as the incidence of isolated DVT (SIR 0.53 (95% CI 0.47–0.60)); conversely, the incidence of PE (±DVT) was unchanged (SIR 1.10 (95% CI, 0.98–1.23)) but the incidence of isolated PE was increased (SIR 1.29 (95% CI, 1.10–1.52)). In 1998, 4.4% of PE cases were diagnosed using CT scan, 31.9% based on the association of proximal DVT and clinical signs of PE, and 62.7% using V/Q scan as

compared with 73.7%, 11.5%, and 14.9% in 2013, respectively ($P < 0.001$).

Conclusion: Between 1998 and 2013, we observed a reduction in the incidence of VTE related to lower rates of DVT; PE incidence remained unchanged.

Disclosure of Interest: None declared.

OR324

Risk factors for venous thromboembolism in women under combined oral contraceptive: the pill genetic risk monitoring (PILGRIM) study

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Background: Identifying women at risk of venous thromboembolism (VTE) under combined oral contraceptives (COC) is a major public health issue.

Aims: To identify determinants of VTE risk in a large cohort of women under COC and to evaluate the accuracy of a Family History Score (FHS) to detect inherited thrombophilia.

Methods: All women with a personal history of VTE under COC and all women under COC with no personal history of VTE addressed to the referral center for thrombophilia in Marseille, France, from 2003 to 2013 were included. Environmental risk factors for VTE were recorded and a thrombophilia screening was performed together with ABO blood group assessment. The FHS was calculated by dividing the number of first-degree relatives who had had an episode of VTE by the total number of first-degree relatives.

Results: One thousand and two cases and 884 controls were included. After adjusting for age, family history, type and duration of COC use, tobacco consumption and Body Mass Index (BMI) were associated with VTE. Odds ratio (OR) was 1.34 (95% confidence interval 1.03–1.76) when consuming 1–14 cigarettes a day (cig day⁻¹), 1.50 (1.03–2.22) between 15 and 24 cig day⁻¹, 2.33 (0.58–12.46) between 25 and 34 cig day⁻¹ and 9.03 (1.98–65.54) beyond. OR was 1.65 (1.23–2.22) for a BMI ≥ 25 and < 30 kg m⁻², 2.90 (1.81–4.79) for a BMI ≥ 30 and < 35 kg m⁻², and 4.12 (2.18–8.34) for a BMI > 35 kg m⁻². In addition, severe heritable thrombophilia (OR = 2.23 [1.39–3.66]) and non-O blood groups (OR = 1.85 [1.49–2.29]) were genetic risk factors for VTE. The FHS had a very low sensitivity (12%) and didn't discriminate severe thrombophilia (% of cases with severe thrombophilia 6.9; 7.3 and 9.3 in FHS ≤ 0.1 ; $0.1 < \text{FHS} \leq 0.3$ and FHS > 0.3 respectively; $P = 0.46$).

Conclusion: A linear relation exists between tobacco consumption, BMI and VTE. This study shows for the first time the impact of ABO blood group on the risk of VTE in women under COC and confirms the inaccuracy of the family history to detect heritable thrombophilia, even using a family history score.

Disclosure of Interest: None declared.

OR325

Splenectomy and risk of venous thrombosis in patients with autoimmune hemolytic anemia (AIHA)

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Background: Splenectomy is efficacious for many patients with AIHA. However, there are concerns about venous thromboembolism (VTE: deep venous thrombosis, pulmonary embolism) and splanchnic vein thrombosis (SVT). Data regarding the incidence and risk factors for these complications has typically been from relatively small studies.

Aims: Determine the effect of splenectomy on the risk VTE and SVT in patients with AIHA.

Methods: We identified a cohort of patients with a diagnosis of AIHA from 1991 to 2011 in the California Discharge Dataset. All records with a procedure code for splenectomy (Spl), or principal or secondary diagnosis of VTE or SVT (portal, hepatic, mesenteric) were identified. Multivariate Cox analysis was performed with VTE or SVT as the outcomes; and age, race, presence of chronic lymphocytic leukemia (CLL) or lupus (SLE), and splenectomy (time-dependent) as covariates.

Results: There were 4658 cases of AIHA. The 5-year cumulative incidences (CI) of VTE and SVT in Spl ($n = 554$) patients were 7.5% and 2.4%, respectively; for non-Spl ($n = 4104$) cases it was 4.9% and 1.0%. Spl was associated with increased risk of both early (< 90 day after operation, hazard ratio [HR] 3.9 CI 2.2–7) and late (> 90 day, HR 2.6 CI 1.8–3.7) VTE; but only early SVT (HR 9.2 CI 3.8–22). Age, CLL, and SLE increased risk and Asian race decreased the risk of VTE. SLE increased the risk of SVT.

Conclusion: In this large cohort of cases with AIHA, splenectomy was associated with significantly increased hazard for both early (within 90 days of operation) and late VTE, whereas splenectomy only increased the short-term risk of SVT. Age, CLL, and SLE increased the risk of VTE. Asians had decreased risk of VTE, but not SVT. These data suggest that increased SVT risk is short-term and related to surgery, whereas there is long-term increased risk for VTE after splenectomy. These data help inform discussions regarding treatment alternatives for AIHA.

Disclosure of Interest: None declared.

Postthrombotic syndrome, thrombophilia

OR326

Post thrombotic syndrome in patients treated with rivaroxaban or enoxaparin/vitamin K antagonists for acute deep vein thrombosis

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Background: Post thrombotic syndrome (PTS) occurs in 20–50% of patients with deep vein thrombosis (DVT). Low quality treatment with vitamin K antagonists (VKA) is one of the risk factors for PTS. Non-vitamin K antagonist oral anticoagulants (NOACs) have a more predictable pharmacologic profile and stable intensity of anticoagulation

than VKA. Hence, we hypothesized that treatment with NOACs may lead to a lower incidence of PTS than VKA.

Aims: To assess the incidence of PTS in rivaroxaban treated patients in comparison to enoxaparin/VKA treated patients.

Methods: *Post hoc* subgroup analysis of the EINSTEIN DVT trial that compared the efficacy and safety of rivaroxaban vs. enoxaparin/VKA for acute DVT. Participating centers were invited to assess PTS after completion of treatment. PTS was defined as a Villalta score of ≥ 5 . Use of elastic compression stockings (ECS) was scored by means of a questionnaire. A chi-square test was performed to compare the PTS incidence between the two treatment groups.

Results: We included 335 patients with a mean age of 58 ± 16 years, 59% male and a median follow-up after index DVT of 57 months (interquartile range 48–64). Of these, 161 (48%) had been treated with rivaroxaban and 174 (52%) with enoxaparin/VKA. Compliance to assigned treatment was $> 80\%$ in 152 (94%) rivaroxaban treated patients vs. 169 (97%) enoxaparin/VKA treated patients and INR was 21% of the time below 2 in the VKA group. PTS occurred in 45 (28%) rivaroxaban treated patients and 66 (38%) enoxaparin/VKA treated patients (RR 0.74; 95% CI 0.54–1.01). ECS were used by 110 (69%) rivaroxaban treated patients vs. 134 (80%) enoxaparin/VKA treated patients.

Conclusion: Rivaroxaban treatment of acute DVT may lower the risk of PTS compared to enoxaparin/VKA treatment. Extrapolating these findings to clinical practice must be done with caution since this was a hypothesis generating post-hoc subgroup analysis. Our findings should be confirmed in adequately sized and designed future studies.

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OR327

Impact of primary venous insufficiency on the prevalence and risk of post-thrombotic syndrome

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Background: Post-thrombotic syndrome (PTS) refers to clinical manifestations of venous insufficiency following a deep vein thrombosis (DVT). The ISTH recommends diagnosing PTS with the Villalta score (VS), which was specifically developed for that purpose. However, studies reported that up to 40% of patients with PTS had also a high (> 4) VS in the contralateral leg (cl VS) that could reflect the presence of pre-existing primary venous insufficiency (PVI) rather than a true PTS. Importantly, in those studies, cl VS was not assessed at time of the DVT event (baseline) and/or patients had a history of previous DVT.

Aims: To estimate the proportion of PTS that could be attributable to pre-existing PVI and the impact of baseline cl VS on the risk of PTS.

Methods: Sub-analysis of the SOX multicentre double-blind RCT of patients with a first symptomatic proximal DVT, no history of DVT and a cl VS assessment at baseline. Patients underwent a bilateral VS assessment at baseline and every 6 months for 2 years. PVI was

defined as a baseline cl VS > 4 and PTS as a VS > 4 in the leg ipsilateral to DVT from 6 months after DVT. We assessed prevalence of PVI in patients who developed PTS and the influence of baseline cl VS on the risk of developing PTS.

Results: Seven hundred and sixty-seven patients were eligible. Mean age was 55 years and 60% were men. During follow-up, 49.1% ($n = 335$) of patients developed PTS, of whom 12.8% ($n = 43$) had a cl VS > 4 (i.e. PVI) at baseline. Baseline PVI and PTS were significantly correlated ($P < 0.0001$). Among 56 patients with PVI at baseline, 76.8% ($n = 43$) developed PTS. The risk of developing PTS increased with increase in cl VS (PTS occurred in 39.6%, 60.4%, 74.1% and 82.4% in patients with a cl VS of respectively 0, 1–4, 5–8, > 8 $P < 0.0001$).

Conclusion: In patients with a first proximal DVT without previous DVT, the proportion of PTS that could in fact reflect pre-existing PVI is modest, but presence and severity of PVI strongly influences the risk of being diagnosed a PTS.

Disclosure of Interest: None declared.

OR328

A simple pediatric post-thrombotic syndrome classification algorithm for the general practitioner

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Background: A simple algorithm for classifying children with or without post-thrombotic syndrome (PTS) can assist general practitioners (GP) determine if patients at risk of PTS require referral to a specialist.

Aims: To develop an algorithm for evaluating children at risk of PTS.

Methods: Four simple methods to measure clinical manifestations [pediatric PTS symptoms questionnaire (PTS_SQ), arm/thigh circumference difference, endurance questionnaire, and Boulden Scale (BS) for collateral veins] were tested in a cross-sectional study of children with upper or lower limb (UL/LL) deep vein thrombosis. A pediatric thrombosis expert independently determined PTS status (PTS+, PTS-). Conditional inference trees (CIT) were developed using unbiased recursive partitioning to assess the predictive value of these four methods for PTS diagnosis. Model performance for classification of PTS+ and PTS- cases was evaluated comparing the predicted (model) vs. actual (expert) PTS diagnosis. Ethics approval and informed consents were obtained.

Results: One hundred and eighteen participants aged 1–19 years were assessed; actual PTS+ frequency was 44% (24/55) for UL and 54% (34/63) for LL.

The UL CIT classified the data according to PTS_SQ and BS. Most children with PTS_SQ score > 2 will be classified as PTS+, whereas most children PTS_SQ score ≤ 2 and BS score = 0 will likely be PTS-. The LL CIT classified the data using PTS_SQ and thigh circumference difference. Most children with PTS_SQ score > 1 will be classified as PTS+, whereas most cases with PTS_SQ score ≤ 1 and thigh difference < 0.9 cm will likely be PTS-.

Both models performed relatively well for classifying PTS+ children: 9–16% of PTS+ children would be misclassified as PTS- (4/24 UL, 3/34 LL). The PTS_SQ alone performed very well for classifying PTS- patients: only 3% of PTS- patients (1/31 UL, 1/29 LL) would be misclassified as PTS+.

Conclusion: The PTS_SQ alone correctly classified 97% of PTS- children. This tool along with the BS and thigh circumference can aid GP caring for children at risk of PTS.

Disclosure of Interest: None declared.

OR329

Hereditary risk factors of thrombophilia and the individual probability of first venous thromboembolism (VTE) in pregnancy and puerperium

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Background: VTE is a leading cause of obstetric morbidity and mortality.

Aims: The aim of our study was to determine the individual probability of thrombosis based on the relative and absolute risk associated with hereditary risk factors of thrombosis.

Methods: In 243 women with first VTE in pregnancy and 243 age-matched women with no history of VTE, RR for hereditary thrombophilic risk factors and the individual probability of thrombosis were determined. Based on epidemiological data, an incidence of VTE of approx. 1:500 deliveries in women > 35 years (approx. 1:750 deliveries in women < 35 years) was assumed.

Results: RR of VTE was 4.6 for heterozygous factor V Leiden (FVL), 20 for homozygous FVL, 3.1 for heterozygous prothrombin gene mutation (PTM), 44 (estimated) for combined heterozygous FVL and PTM, 2.0 ($P = 0.083$) for antithrombin (AT) deficiency < 90% (10th percentile) and 49 for < 60%, 2.3 for protein C deficiency < 76% (10th percentile) and 5.5 for < 50%, 4.1 for protein S deficiency < 40% ($P = 0.089$), and 9.7 for free protein S deficiency < 40% ($P < 0.05$ for all unless noted otherwise).

In women > 35 years/< 35 years, the probability of pregnancy-related thrombosis was 0.73%/0.48% for heterozygous FVL, 3.9%/2.6% for homozygous FVL, 0.58%/0.38% for heterozygous PTM, 7.8%/5.3% for combined heterozygous FVL and heterozygous PTM, 0.36%/0.26% for AT deficiency < 90%, and 9.1%/6.1% for < 60%, 0.49%/0.26% for protein C deficiency < 76%, and 1.1%/0.75% for < 50%, 0.72%/0.54% for protein S deficiency < 40%, and 2.02%/1.3% for free protein S deficiency < 40%. 7 of 17 women with VTE and homozygous or combined heterozygous FVL/PTM had no first-degree relatives with a history of VTE.

Conclusion: We could show that women with homozygous or combined heterozygous FVL/PTM or with AT deficiency have a high risk of pregnancy-associated thrombosis, particularly women aged > 35 years. This subgroup should be considered for routine antepartum thromboprophylaxis even with a negative family history of VTE in future guidelines.

Disclosure of Interest: None declared.

OR330

Clinical and genetic features of antithrombin deficiency in 13 Taiwanese patients

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Background: Inherited antithrombin (AT) deficiency accounts for 0.5–4.9% in patients with venous thromboembolism (VTE) and is associated with a 5–50 fold increased risk for VTE. There were very limited cases reported from Taiwan previously.

Aims: This study was to analyze the clinical presentations and genetic defects of 13 patients with inherited AT deficiency associated VTE and thrombophilia.

Methods: Thirteen patients from eleven unrelated families were diagnosed to have inherited AT deficiency associated VTE from August 2009 to April 2014. There were 9 male and 4 female patients. Their median age was 40 years old with a range of 16–46 years. Their clinical manifestations were recorded and peripheral blood DNA was extracted. The 6 encoded exons of AT gene were amplified by polymerase chain reaction, followed by direct sequencing.

Results: The median age of first VTE onset was 25 years old with a range of 5–44 years old. The mean AT activity of these patients was $43.03 \pm 5.88\%$. Seven of the 13 (54%) had developed deep venous thrombosis (DVT) of lower extremities as initial presentation, Seven patients (54%) had experienced pulmonary embolism (PE). There were each two patients with mesenteric vein thrombosis, portal venous thrombosis events and CNS thrombosis, respectively. The genetic defects of the 11 unrelated families were all heterozygous mutations, including 6 missense, 3 deletion and 2 insertion. Eight of them are novel and have not been reported before.

Conclusion: Our study demonstrates one half of anti-thrombin (AT) deficiency patient presented with deep vein thrombosis as initial presentation. One half patients experienced PE and 20% of patients had portal vein thrombosis and mesenteric venous thrombosis, respectively. Eight novel AT gene defects were found.

Disclosure of Interest: None declared.

Venous Willebrand factor – I

OR331

Phage display and next generation DNA sequencing enables high resolution mapping of VWF functional domains and alloantibody epitopes

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Background: von Willebrand factor (VWF) is a large multimeric protein composed of multiple, distinct functional domains. The substructures of these domains are important in regulating VWF function and for epitope recognition by anti-VWF inhibitors.

Aims: Fine map the VWF platelet-binding domain and characterize epitopes recognized by an acquired VWF inhibitory antibody.

Methods: An M13 filamentous phage library was constructed, expressing random VWF cDNA fragments and consisting of $\sim 2.8 \times 10^6$ independent clones. The library was screened for binding to formalin-fixed platelets or to plasma immunoglobulins isolated from a type 3 von Willebrand disease patient with acquired an inhibitor. In both screens, bound phage were eluted following a single round of selection and analyzed by next generation DNA sequencing (NGS). Approximately 10^6 – 10^7 reads were processed for alignment to VWF and peptide identification.

Results: Analysis of aligned reads mapped the platelet binding segments to two regions in VWF. Maximally enriched residues (A1250-A1461) spanned the A1 domain. Fragments spanning the A1 domain all encompassed C1272-C1458, indicating that disulfide bridging of these cysteines optimizes platelet binding. A weaker signal in the D4 domain (D1983-I2126) suggested a potential, secondary adhesive region. Surveying for epitopes of the patient's VWF inhibitors by immunoprecipitation and NGS found strongly enriched clusters of residues that spanned the A3 (collagen binding) domain (maximally,

I1677-C1872) and encompassed the C1686-C1872 disulfide bridge. A cluster of residues (A751-H874) with weaker signals was also detected in the VWF D' (FVIII binding) domain; these residues encompassed all of D' (S764-A865). The absence of smaller fragments suggests that this alloantibody recognized only these two conformational epitopes.

Conclusion: Phage display combined with NGS provides a versatile and powerful tool for defining functional domains and antibody epitopes within VWF.

Disclosure of Interest: None declared.

OR332

Scavenger receptor class A member 5 (SCARA5) binds and internalizes VWF *in vitro*: a novel candidate VWF clearance receptor

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Background: Details of the VWF clearance mechanism are still unresolved. A recent genome-wide association study (GWAS) revealed the locus for the scavenger receptor class A member 5 (SCARA5) as being a significant genetic modifier of VWF levels. SCARA5 is a cell surface receptor having similar domain structure to SCARA1 (SR-A) or SCARA2 (MARCO). Previous studies of SCARA5 have documented its expression on interstitial fibroblasts but not on endothelial or blood cells, while SR-A and MARCO were expressed on macrophages.

Aims: To explore if SCARA5 is a candidate clearance receptor for VWF through complementary molecular and cell biology studies.

Methods: Binding of VWF to SCARA5 was assessed using a surface plasmon resonance assay (SPR) and a solid phase binding assay. Binding of VWF to SCARA5-expressing HEK 293 cells was evaluated with immunofluorescence (IF). Association of SCARA5-expressing cells with VWF in the spleen and liver was evaluated in VWF^{-/-} mice infused with human VWF by immunohistochemistry (IHC).

Results: The binding of human plasma-derived VWF to immobilized SCARA5 was dose-dependent and saturable with an apparent K_d of ~1 nM. Ca²⁺ chelation with EDTA impaired the binding of VWF to SCARA5 (> 90%), while preincubation of VWF with the polyanion PolyI attenuated VWF binding to SCARA5 by ~10% suggesting the Ca²⁺-dependent binding property. Recombinant human FVIII bound SCARA5 in the presence, but not the absence, of VWF. IF demonstrated that human VWF was bound and internalized by transfected HEK 293 cells expressing SCARA5 but not by untransfected cells. IHC confirmed that infused human VWF was mostly associated with endothelial cells and macrophages in the liver and spleen. However, co-localization of SCARA5-expressing cells and infused human VWF has not been observed with current experimental conditions.

Conclusion: SCARA5 is an adhesive and endocytic receptor for VWF in solid phase and cell based assays. However, the mechanism by which SCARA5 and VWF may interact *in vivo* remains to be investigated.

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OR333

New *in vitro* and *in vivo* strategies to modulate von Willebrand factor gene mutations with dominant-negative effect

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Background: von Willebrand factor (VWF) is a multimeric protein that undergoes dimerization and multimerization processes during its biosynthesis. Dominant-negative mutations are associated with severe von Willebrand disease phenotypes but remain difficult to identify.

Aims: To study dominant-negative mutations and their molecular mechanisms.

Methods: We created *in vitro* and *in vivo* transient models to express VWF carrying the p.P1127_C1948delinsR (del) and p.C2773R dominant-negative mutations affecting multimerization and dimerization processes, respectively. COS-1 cells were transiently co-transfected with plasmids for wild-type (wt) and mutant VWF expression. Similarly, Vwf-deficient mice were subjected to hydrodynamic gene transfer of both plasmids. VWF multimers were evaluated in conditioned media and mouse plasma.

Results: As expected, co-expression of pC2773R- with wt-VWF was associated with absence of high molecular weight multimers but normal VWF antigen levels in conditioned media.

Co-expression of del- & wt-VWF also resulted in severe reduction of VWF multimers and high-resolution gel enabled the separation of heteropolymers formed by wt- and del-VWF subunits. We next created plasmids for the expression of VWF carrying both gene defects (p.del/C2773R) in cells and mice. Noticeably the double-mutant VWF was unable to interact with wt monomers leading to abolition of the dominant-negative effect of the single defects and rescue of the multimer profile.

The detrimental effect of the large deletion was also challenged *in vivo* by the administration of siRNA selectively directed against del-VWF. By interfering with the dominant-negative mechanism, the silencing treatment improved VWF antigen levels and multimer profile.

Conclusion: We established the first *in vivo* heterozygous mouse model of VWD associated with dominant-negative mutations. The association of other VWF mutations with del-VWF can be employed to investigate/antagonize their dominant-negative effect.

Disclosure of Interest: None declared.

OR334

Role of calcium in regulating the intra- and extra-cellular cleavage of von Willebrand factor by the metalloprotease ADAMTS13

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Background: The metalloprotease ADAMTS13 cleaves a scissile bond in the von Willebrand Factor (VWF) A2-domain in a calcium dependent manner. The physiological relevance of this calcium dependence remains unknown.

Aims: Since VWF and ADAMTS13 are co-expressed in endothelial cells (EC), we hypothesized that the low intra-cellular calcium levels (Ca²⁺ = 80–400 μM) may promote VWF cleavage prior to protein secretion.

Methods: A panel of recombinant VWF proteins were expressed in HUVECs (Human Umbilical Vein Endothelial Cells) and control HEK cells, sometimes over-expressing ADAMTS13. VWF proteolysis

was measured using western blot; A2-domain conformation change was quantified using FRET constructs where Venus and Cerulean flanked VWF-A2; and platelet string cleavage under shear was studied using microfluidic flow cells.

Results: The results demonstrate the partial perinuclear colocalization of VWF with ADAMTS13 in HUVECs, and the presence of VWF proteolysis products in both HUVEC lysates and culture-supernatant following EC stimulation. Both wild-type multimeric VWF (WT-VWF) and VWF with a disulfide link bridging the N- and C-terminus of VWF-A2 (Lock-VWF) displayed equal intracellular cleavage when expressed in HUVECs. Thus, ADAMTS13 mediated proteolysis occurs prior to disulfide bond formation. Two constructs that have defective calcium binding epitopes, the VWD type 2A mutant R1597W and D1498A, displayed enhanced intracellular proteolysis in HUVECs compared to WT-VWF. The FRET studies demonstrate strong Ca^{2+} dependent VWF-A2 conformation changes at concentrations $< 500 \mu\text{M}$, with unfolding rates being ~ 40 -fold higher for the monomeric VWF A2-domain compared to multimeric, full length VWF. Further, VWF-platelet string cleavage under shear proceeded with equal efficiency in the absence and presence $1.5 \text{ mM } \text{Ca}^{2+}$.

Conclusion: Low Ca^{2+} may promote intracellular VWF proteolysis. Extracellular VWF-platelet string proteolysis is Ca^{2+} independent.

Disclosure of Interest: None declared.

OR335

Role of protein disulfide isomerase pdi in von Willebrand factor dimerization

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Background: Von Willebrand factor (VWF) is a multimeric glycoprotein essential for primary hemostasis. The biosynthesis of VWF high molecular weight multimers requires spatial separation of each step due to varying pH value requirements. The multimerization occurs in the acidic environment of the trans-Golgi apparatus. VWF is dimerized by disulfide bonds between the CK domains of two VWF monomers, formed at neutral pH in the ER. Which protein catalyzes this C-terminal disulfide bond formation has not been elucidated yet.

Aims: The protein disulfide isomerase PDI has previously been used to visualize colocalization of VWF with the ER. However, if these two proteins are indeed binding partners and the reason for this putative direct interaction have never been investigated in detail.

Methods: We used STORM and confocal microscopy, Microscale Thermophoresis (MST), Fluorescence Correlation Spectroscopy (FCS) and protein-protein docking studies to characterize PDI-VWF interaction and postulate a dimerization mechanism for VWF.

Results: Excitingly, we were able to visualize the interaction of PDI with VWF employing high-resolution STORM microscopy. MST and FCS clearly showed direct binding of PDI to VWF. The dissociation constants were determined to be $K_D = 236.0 \pm 66 \text{ nM}$ and $K_D = 282.4 \pm 123 \text{ nM}$ by MST and FCS, respectively. Since the interaction of PDI and the isolated CK domain exhibits a similar K_D value ($258 \pm 104 \text{ nM}$), our data indicate that PDI binds exclusively to the CK domain. Fluorescence microscopy studies of VWF mutants associated with von Willebrand disease 2A/IIID, that exhibit a disturbed VWF dimerization, revealed altered PDI interaction depending on the position of the mutation.

Conclusion: Combined with protein docking studies we postulate the mechanism of VWF dimerization by PDI. Our data indicate that Cys2771 is the essential initiation point of dimerization that begins with the formation of the two disulfide bonds 2771-2773' and 2771'-2773. Subsequently, the third bond 2811-2811' is formed.

Disclosure of Interest: None declared.

Platelet disorders – bleeding

OR336

Defective $\alpha\text{IIb}\beta_3$ activation causes platelet dysfunction in von Willebrand disease platelet-type (PT-VWD)

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Background: PT-VWD is an autosomal dominant hemorrhagic disorder with mucocutaneous bleeding and macrothrombocytopenia, due to gain-of-function mutations in *GPIBA* conferring to platelet glycoprotein $\text{Ib}\alpha$ ($\text{GPIb}\alpha$) enhanced affinity for von Willebrand factor (VWF). The bleeding tendency is generally considered to be due to thrombocytopenia and reduction of high molecular weight VWF multimers for the clearance of VWF-platelet complexes from the circulation. On the other hand, the binding of VWF to $\text{GPIb}\alpha$ is known to trigger intracellular signaling leading to $\alpha\text{IIb}\beta_3$ activation. Defective $\alpha\text{IIb}\beta_3$ activation was described in a mouse model of PT-VWD.

Aims: Aim of this work was to shed new light on the effects of the enhanced $\text{GPIb}\alpha$ -VWF interaction on $\alpha\text{IIb}\beta_3$ activation and platelet function in PT-VWD.

Methods: Platelets from a PT-VWD patient carrying the Met239Val *GPIBA* mutation were studied for aggregation and shape change by light transmission aggregometry; for $\alpha\text{IIb}\beta_3$ expression and activation (PAC-1 binding), Ca^{2+} store release and α -granules secretion by flow cytometry; for δ -granules secretion by lumiaggregometry; moreover, platelet spreading on fibrinogen and VWF was assessed, and Rap-1b activation (Rap-1b-GTP), a crucial step for $\alpha\text{IIb}\beta_3$ activation, was measured by Western blotting.

Results: Platelets from PT-VWD show defective $\alpha\text{IIb}\beta_3$ activation after stimulation with ADP or TRAP-6. Platelet aggregation in response to TRAP-6 was strongly reduced, second wave of aggregation after stimulation with epinephrine was absent and shape change triggered by several agonists was absent. Granules secretion was normal but platelet spreading was impaired, suggesting defective $\alpha\text{IIb}\beta_3$ -mediated outside-in signaling. Ca^{2+} store release, required for $\alpha\text{IIb}\beta_3$ activation, was normal but Rap-1b activation was reduced.

Conclusion: Our data show for the first time that $\alpha\text{IIb}\beta_3$ activation and function are impaired in human PT-VWD. These data set the basis for a full understanding of the bleeding tendency in PT-VWD.

Disclosure of Interest: None declared.

OR337

Comprehensive evaluation of mechanisms associated with hyper-responsive platelet GPIbA and the role of protein inhibition in securing hemostasis

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Background: Platelet defects due to hyper-responsive $\text{GPIb}\alpha$ causing an enhanced VWF interaction, counter-intuitively result in bleeding rather than thrombosis. The historical explanation of platelet/VWF

clearance fails to explain mechanisms of impaired hemostasis particularly in light of reported poor platelet binding to fibrinogen. Platelet transfusion carries risk for developing alloantibodies.

Aims: To study the pro-coagulant capacity of platelets with hyper-responsive GPIIb/IIIa, kinetics of thrombus formation including clot quality and the effect of GPIIb/IIIa inhibition on securing hemostasis.

Methods: Platelets from PT-VWD mouse model were compared to control mice. Global hemostasis and clot kinetics were assessed *in vitro* using thromboelastography (TEG) and *in vivo* using intravital microscopy. ADP and thrombin-induced whole blood platelet aggregation were tested. P-selectin and PS exposure were analyzed using flowcytometry. Fibrin clot quality was evaluated in standard fibrin polymerization technique and fibrin structure was examined using confocal microscopy. 6B4; monoclonal antibody that blocks interaction with VWF was used for GPIIb/IIIa inhibition.

Results: TEG showed 23% increase in R-time, 39% reduction in MA, 74% reduction in CI in Mut compared to WT mice. *Ex-vivo* inhibition of GPIIb/IIIa normalized R and MA by 33% and 41% respectively and *in vivo* inhibition enhanced clot formation in intravital data. Annexin V expression was reduced by 76, 42 and 57% when PRP was stimulated with thrombin, collagen and ristocetin respectively. P-selectin expression was markedly reduced in response to thrombin. Platelets aggregated normally to thrombin but showed long lag phase. Turbidity assay showed delayed fibrin formation which was of low density under confocal microscope.

Conclusion: Platelets with hyper-responsive GPIIb/IIIa have complex pro-coagulant properties that reflect intrinsic impairment beyond the previously described mechanisms. Abnormal signaling through GPIIb/IIIa and potential therapy using inhibitors require investigation.

Disclosure of Interest: None declared.

OR338

Oxidative stress accelerates platelet receptor glycoprotein GPIIb/IIIa and GPVI shedding during non-surgical bleeding in heart failure patients supported by continuous-flow left ventricular assist device

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Background: Understanding the role of oxidative stress in modifying platelet functionality contributing to non-surgical bleeding (NSB) in heart failure (HF) patients after continuous-flow left ventricular assist device (CF-LVAD) implantation is crucial for prevention of this adverse event.

Aims: To examine the role of oxidative stress in mediating platelet receptors shedding on the incidence of NSB after CF-LVAD implantation.

Methods: We recruited 25 HF patients implanted with CF-LVADs and 11 healthy volunteers as control. Eight HF patients developed non-surgical bleeding (bleeder group) within one month after implantation while others were considered non-bleeder group ($n = 17$). Generation of reactive oxygen species (ROS) in leukocytes and platelets, status of antioxidant enzyme superoxide dismutase (SOD) in erythrocyte, total antioxidant capacity (TAC), oxidized low density lipoprotein (oxLDL) and the surface expression of platelet receptor glycoproteins GPIIb/IIIa and GPVI were measured.

Results: Generation of ROS, depletion of SOD and TAC, and elevated oxLDL were found to be pre-existing conditions in baseline samples of both groups of HF patients when compared to healthy controls. Platelet receptors GPIIb/IIIa and GPVI were found to be significantly lowered

in bleeder group prior to CF-LVAD implantation in comparison to non-bleeder group indicating shedding. Significant increase in intraplatelet ROS and decrease in GPIIb/IIIa and GPVI were noticed in post-implant bleeder group in comparison to non-bleeder group. Significant decrease in post-implant GPIIb/IIIa and GPVI in bleeder group were positively associated with ROS and oxLDL, and negatively associated with SOD and TAC suggesting the potential role of oxidative stress in platelet receptor shedding and mediating NSB after CF-LVAD implantation.

Conclusion: In conclusion, persistent oxidative stress may play a potential role in accelerating platelet receptor glycoprotein GPIIb/IIIa and GPVI shedding leading to platelet dysfunction during NSB in HF patients supported by CF-LVADs.

Disclosure of Interest: None declared.

OR339

Methods for identifying rare genetic variants affecting the formation and function of platelets

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Background: The BRIDGE-BPD consortium is a multi-centre collaboration working to identify novel genetic causes of inherited bleeding and platelet disorders (BPD). We have performed detailed phenotyping, pedigree building and genome sequencing of approximately 1000 cases. The extensive but often subtle clinical heterogeneity of cases has been encoded using Human Phenotype Ontology (HPO) terms.

Aims: The discovery of novel causal variants affecting the formation and function of platelets.

Methods: Detailed phenotyping, pedigree building and genome sequencing of approximately 1000 cases in combination with the development of new statistical methods to aid gene discovery.

Results: We have developed new statistical approaches to perform inference using genetic and HPO data. First, we have developed a method to assess the phenotypic distinctness of an arbitrary group of cases based on their HPO terms. If the groupings are based on genotypes, highly distinct groups are likely to share a common disease aetiology. Second, we have used HPO and Mouse Phenotype Ontology (MPO) terms from the literature to prioritise candidate genes based on small lists of filtered variants. Third, we have developed a novel 'similarity regression' algorithm to find associations between rare variants and a BPD whilst inferring the primary phenotypic features of the particular disease. With the aid of the above approaches, we have discovered and subsequently confirmed genes causative of BPDs at 20q11 and 5q31. In addition, these approaches have helped replicate recent discoveries by others that variants in RASGRP2 (11q13.1) and ACTN1 (14q24.1) can be causative of BPD. Such discoveries improve our understanding of the molecular basis of this class of disorders and the newly discovered genes have been included on the ThromboGenomics diagnostic sequencing platform.

Conclusion: The development of new methods that exploit the rich structure of HPO and MPO empowers gene discovery in heterogeneous case collections with diverse molecular aetiologies.

Disclosure of Interest: None declared.

OR340

Severe bleeding tendency and impaired platelet function in a patient with CalDAG-GEFI deficiency

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Background: Platelets are essential in normal hemostasis and pathological thrombosis, and their function is tightly regulated in circulation. Study of patients with bleeding disorders of platelet function provides quite important clue to find a signaling pathway and molecules required for platelet activation.

Aims: To investigate the cause of bleeding problem in 15-year-old Japanese girl.

Methods: The proband has been suffering from repeated severe spontaneous nasal bleeding which required transfusions since she was 1 year old. The initial laboratory assessment indicated normal platelet count, normal PT/APTT, markedly prolonged bleeding time, and reduced platelet aggregation responses to ADP and collagen. Recently she started suffering from menorrhagia and she was referred to our hospital. Peripheral bloods obtained from the proband and her parents were analyzed by flow cytometry, Western blotting, and sequencing. Written informed consent was obtained from all subjects.

Results: Expression levels of her platelet surface glycoproteins were comparable to those of control. Although PMA-induced integrin α IIB β 3 activation was normal, α IIB β 3 activation induced by various agonists was impaired. Platelet granule release was also decreased. In addition, slower α IIB β 3 activation kinetics was observed by 'initial velocity assay'. These results, along with normal calcium mobilization and impaired Rap1 activation, suggest impaired activation process of α IIB β 3. Western blotting revealed the deficiency of CalDAG-GEFI, but normal talin and Kindlin-3 expression in her platelets. Sequencing results revealed compound heterozygous mutation, R360del and K309X, in CalDAG-GEFI. Introduction of expression vector for mutant CalDAG-GEFI into 293T cells confirmed that these mutations are responsible for the deficiency of CalDAG-GEFI.

Conclusion: We identified new mutations of CalDAG-GEFI causing severe bleeding problem. Our results indicate the essential role of CalDAG-GEFI in α IIB β 3 activation as well as hemostatic function of platelets.

Disclosure of Interest: None declared.

Platelets and cancer

OR341

15(S)-HETE is a pro-angiogenic factor produced by platelets through COX-1

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Background: Thromboxane A₂ (TXA₂) is a potent pro-thrombotic hormone produced at high concentrations by platelets through the activity of cyclooxygenase-1 (COX-1). Aspirin, by inhibiting COX-1, reduces TXA₂ levels which explains its use for secondary prevention of vascular thrombotic events. Epidemiological and observational analyses also demonstrate that chronic use of low-dose aspirin is associated

with a reduction in risk for certain types of cancer, consistent with the anti-angiogenic properties of this drug.

Aims: Here we have identified 15-hydroxyeicosatetraenoic acid (15(S)-HETE) as a major COX-1 product of platelets and investigated its role in angiogenesis.

Methods: Blood from healthy volunteers was incubated with aspirin either intact or as platelet rich plasma (PRP) prior to stimulation with thrombin receptor-activating peptide-6 (TRAP-6) or vehicle. Plasmas were separated and the levels of TXB₂ (TXA₂ marker) and 15-HETE were determined by LC-MS/MS, and those of the 15(S)-HETE stereoisomer by EIA assay. The angiogenic properties of 15(S)-HETE were studied by assessing tube formation of HMEC-1 cells and sprout formation by rat aortas incubated with plasmas obtained from TRAP-6-stimulated platelets pre-treated with or without aspirin.

Results: TRAP-6 caused marked increases in the platelet productions of TXA₂ and 15-HETE both of which were strongly inhibited by aspirin. Treatment of platelets with aspirin also strongly reduced the pro-angiogenic effects of platelet plasmas in both the endothelial tube formation and rat aorta sprouting models. Angiogenic responses were fully restored by addition of exogenous 15(S)-HETE.

Conclusion: 15(S)-HETE is a major platelet COX-1 product which, in association with other growth factors, has strong pro-angiogenic effects. Thus, 15(S)-HETE represents a potential target for the development of novel anti-angiogenic therapeutics.

Disclosure of Interest: None declared.

OR342

Platelets engineered to store interleukin-24 inhibited melanoma growth in mice

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Background: Activated platelets secrete agents (e.g., cytokines) that can promote solid tumor growth & cancer metastasis. Previous studies showed hematopoietic stem cells (HSC) can be genetically modified to induce platelets to express & secrete proteins to establish hemostasis in animal models of bleeding disorders. Interleukin-24 (IL-24) is a cytokine (normally produced by activated monocytes, macrophages & T-helper cells) with cytotoxic & anti-angiogenic activity preferentially towards cancer cells. Thus, we hypothesized it may be feasible use HSC gene transfer to target platelet synthesis, storage & secretion of IL-24 to inhibit tumor growth.

Aims: To investigate if HSC gene transfer targeting IL-24 synthesis, storage & secretion from platelets can inhibit melanoma in mice.

Methods: C57BL/6 mice were transplanted with bone marrow transduced with a lentiviral construct encoding a megakaryocyte-specific *ITGA2B* gene promoter driving synthesis of IL-24 gene. Four weeks after transplant, IL-24 protein was characterized in platelets by flow cytometry. Murine melanoma cells (1×10^6) were implanted in mice at 5 weeks after transplant. Tumor size was measured in IL-24 & control groups using a digital caliper periodically for 4 weeks. Then mice were sacrificed to record tumor mass.

Results: Flow cytometry showed that HSC gene transfer of murine bone marrow led to synthesis & storage of IL-24 in platelets. At 30 days after implant of melanoma cells, platelet IL-24 mice displayed significantly smaller ($\approx 50\%$) tumor size ($550 \pm 101 \text{ mm}^3$) & weight ($599 \pm 120 \text{ mg}$) compared to control mice with larger tumor size ($1120 \pm 114 \text{ mm}^3$) & weight ($1391 \pm 134 \text{ mg}$) $n \approx 5$ mice/group ($P < 0.05$) indicating that platelet IL-24 inhibited tumor growth *in vivo*.

Conclusion: HSC gene transfer can be utilized to induce synthesis & storage of anti-oncogenic agent IL-24 in platelets. Melanoma tumor challenge in mice with platelet IL-24 showed a significant decrease in

tumor growth suggesting that platelets may serve as a therapeutic vehicle to treat cancer.

Disclosure of Interest: None declared.

OR343

CLEC-2 facilitates hematogenous tumor metastasis and *in vitro* tumor growth, but not *in vivo* tumor growth

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Background: We reported that a platelet activation receptor, C-type lectin like receptor-2 (CLEC-2) facilitates hematogenous metastasis by binding to podoplanin, a membrane protein of tumor cells in ISTH 2013. We also reported that hematogenous metastasis of podoplanin-positive B16F10 melanoma, but not tumor growth, was inhibited in CLEC-2 $-/-$ bone marrow chimeric mice, although tumor cell proliferation *in vitro* was facilitated by platelets depending on CLEC-2.

Aims: The aim of this study is to investigate why tumor growth *in vivo* is not inhibited in the absence of CLEC-2, although *in vitro* proliferation is increased by platelets depending on CLEC-2.

Methods: CLEC-2-depleted mice were generated by injection of anti-CLEC-2 antibody, 2A2B10 every 7 days. 1×10^6 cells of B16F10 was inoculated into the dorsal skin of mice 4 days after 2A2B10 or control IgG injection. Tumor growth was evaluated by measuring the size of tumors. Intra-tumor angiogenesis and thrombosis were analyzed by immunohistochemistry. Intra-tumor functional vessels were determined by injection of FITC-dextran from the tail vein before sacrifice. Survival was analyzed by Kaplan–Meier method.

Results: Tumor growth was not inhibited in CLEC-2-depleted mice and the number of intra-tumor vessels did not differ between CLEC-2-depleted and control mice. However, the number of functional vessels in tumors significantly increased in CLEC-2-depleted mice. Intra-tumor thrombus formation was decreased in CLEC-2 depleted mice. Unexpectedly, CLEC-2-depleted mice exhibited prolonged survival.

Conclusion: We suggest that CLEC-2 depletion results in decrease in intra-tumor thrombus formation, which leads to increase in intact tumor vessels and efficient supply of oxygen and nutrition to tumor cells *in vivo*. As a result, the growth-stimulating effect of platelets via CLEC-2 observed *in vitro* is canceled. Less thrombotic tendency may be related to prolonged survival, although the cause for this finding is now under investigation.

Disclosure of Interest: None declared.

OR344

Identification of a novel C-type lectin-like receptor 2 inhibitor that suppresses podoplanin-induced platelet aggregation and cancer metastasis

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Background: The binding of podoplanin (PDPN) to C-type lectin-like receptor 2 (CLEC-2) is crucial in platelet activation, lymphogenesis and cancer metastasis. Blockage of CLEC-2 signaling is a rationale scheme for development of anti-cancer metastasis and anti-thrombosis regimens.

Aims: We aim to identify synthetic compound to intervene CLEC-2 signaling. The mechanistic insight and the potential application of the compound were investigated.

Methods: A pool of synthetic compounds was examined for their effects on agonists-induced platelet aggregation. The compound that shows specific inhibition on PDPN-induced platelet aggregation were selected for characterizing the mechanistic insight using phospho-specific antibodies, protein kinase profiling, molecular modeling and surface plasmon resonance analyses. The anti-cancer metastasis efficacy of the compound was evaluated by tumor cell-induced platelet aggregation (TCIPA) assay and measuring tumor metastasis in a xenograft mouse model.

Results: A compound 2CP was identified to selectively inhibit PDPN-but not other agonists-induced platelet aggregation ($IC_{50} = 12.1 \mu M$). The activities of all CLEC-2 downstream signaling proteins examined, including the newly identified mediators Akt1/PDK1 and PKC μ , were all affected by 2CP. Nevertheless, 2CP did not inhibit the activities of 25 selected protein kinases that play a role in platelet signaling. 2CP thereby does not appear to directly act on the cytoplasmic signaling proteins. Instead, 2CP bound CLEC-2 ($K_d = 33.2 \mu M$) and competed for the same PDPN binding residues of R107, R118, R152 and R157 in CLEC-2. 2CP was further defined to possess anti-cancer metastatic activity by impeding PDPN-mediated TCIPA and augmented the therapeutic efficacy of cisplatin. Pulmonary tumor foci formation and the body weight loss associated with the increase in tumor burden were both attenuated by cotreatment of 2CP and cisplatin.

Conclusion: 2CP is the first defined CLEC-2 inhibitor with potential for developing anti-cancer metastasis regimens.

Disclosure of Interest: None declared.

OR345

Survival after cancer-associated venous thrombosis: results from the Scandinavian Thrombosis and Cancer (STAC) study

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Background: Cancer patients have an increased risk of venous thrombosis. Those cancer patients who develop a venous thrombotic event (VTE) are reported to have poor survival.

Aims: To investigate the impact of venous thrombosis on survival of cancer patients in a general population.

Methods: We used data from the Scandinavian Thrombosis and Cancer (STAC) cohort, a population-based cohort including 144,952 subjects without previous VTE or cancer. During follow-up, incident cancer and VTE were registered. 'Cancer-related VTE' was defined as VTE diagnosed in patients with overt or occult cancer (i.e., cancer diagnosed within 1 year after VTE). Survival of subjects without cancer or VTE was compared with survival of subjects diagnosed with cancer and cancer-related VTE during follow-up. Cox-regression models with cancer and VTE as time-varying exposure were performed to calculate hazard ratios (HR) for death. Sub-analyses were performed across various cancer types and stages and type of VTE [i.e., deep vein thrombosis (DVT) or pulmonary embolism (PE)].

Results: During follow-up (mean 11.6 years) 14,621 subjects developed cancer and 2444 VTE. There were 567 cancer-related VTEs (454 with overt, 113 with occult cancer). The mortality rates (per 100 person years) for patients without VTE and without cancer, with VTE only, with cancer only and with cancer-related VTE were 0.63 (95% CI 0.62–0.64), 5.0 (95% CI 4.5–5.5), 11.6 (95% CI 11.3–11.9) and 45.3 (95% CI 41.0–50.0), respectively. Compared with subjects without VTE and cancer, the HR of death for patients with cancer only was 9.4 (95% CI 9.1–9.8), while it was 25.9 (95% CI 23.4–28.6) for patients with cancer and VTE. This association was seen across all cancer types. Cancer patients with related DVT had a higher risk of death than cancer patients with PE (HR 28.7, 95% CI 25.2–32.7 vs. 22.4, 95% CI 18.8–26.7).

Conclusion: In a general population, cancer patients with VTE had a much higher risk of death than patients with cancer only.

Disclosure of Interest: None declared.

Hemophilia B

OR346

Efficacy, pharmacokinetics (PK) and safety results of a phase 3 clinical study of recombinant fusion protein linking coagulation factor IX with albumin (RIX-FP) in previously treated children with hemophilia B

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Background: A fusion protein genetically linking recombinant human coagulation FIX with recombinant human albumin (rIX-FP) has been developed with an improved PK profile, thus improving hemophilia B treatment by allowing less frequent dosing.

Aims: In this study in the PROLONG-9FP program, the long-term safety and efficacy of rIX-FP were evaluated for both prophylaxis and on-demand treatment of bleeding episodes in children for 12 months and 50 exposure days (EDs).

Methods: Subjects were previously treated male patients < 12 years (y) old with hemophilia B (FIX ≤ 2%) and they received weekly prophylaxis and on-demand treatment for bleeding episodes. Annualized spontaneous bleeding rates (AsBR) were calculated. Treatment efficacy was evaluated by number of injections to achieve hemostasis and Investigator assessment. PK of rIX-FP and previous FIX product were conducted at the start of the study.

Results: A total of 27 subjects enrolled; 12 were < 6 years. rIX-FP had an improved PK profile including > 5-fold longer half-life compared with previous FIX products, supporting a prophylaxis treatment interval of every 7–14 days. The median (Q1, Q3) AsBR was 0.00 (0.00, 0.91) and was similar between < 6 and ≥ 6 years age groups, with weekly median prophylaxis dose of 46 IU kg⁻¹. Overall 97% of bleeding episodes were successfully treated with 1 or 2 injections of rIX-FP (95% CI: 92% to 99%), and 96% of the treatments were rated effective (excellent or good) by the Investigator. A total of 25/27 subjects achieved ≥ 50 EDs. No subjects developed inhibitors to FIX or antibodies to rIX-FP. There were no related AEs and no withdrawals from the study.

Conclusion: This phase 3 study demonstrated the clinical efficacy of rIX-FP for weekly routine prophylaxis and treatment of bleeding epi-

sodes in children. A prophylaxis interval of 14-days is viable option and will be offered in the extension study. In addition, rIX-FP had a favorable long-term safety and tolerability profile, with no inhibitors detected.

Disclosure of Interest: G. Kenet Grant/Research Support from: BPL, Baxter, Prolor Biotech, Consultant for: Prolor Biotech, Bayer, Paid Instructor at: Pfizer, Novo Nordisk, Speaker Bureau of: Bayer, H. Chambost Grant/Research Support from: CSL Behring, Novo Nordisk, LFB, Consultant for: CSL Behring, Novo Nordisk, Baxter, Bayer, Pfizer, Speaker Bureau of: Novo Nordisk, LFB, C. Male Speaker Bureau of: CSL Behring, T. Lambert: None declared, M. Álvarez-Román Consultant for: Baxter, Bayer, NovoNordisk, Pfizer, S. Halimeh: None declared, A. Chan: None declared, C. Barnes: None declared, T. Chernova: None declared, J. Blatny Consultant for: Baxter, Speaker Bureau of: Baxter, Bayer, NovoNordisk, M. E. Mancuso Consultant for: CSL Behring, S. Meunier: None declared, V. Komrska: None declared, H.-J. Laws Consultant for: CSL Behring, Paid Instructor at: GSK, AstraZeneca, Pfizer, NovoNordisk, M. Morfini Grant/Research Support from: Bayer, Pfizer, Baxter, Consultant for: Bayer, Baxter, NovoNordisk, Pfizer, Speaker Bureau of: CSL Behring, Biotest, Bayer, NovoNordisk, J. Curtain Grant/Research Support from: CSL Behring, Consultant for: CSL Behring, Baxter, Biogen Idec, NovoNordisk, B. Blazek: None declared, C. Voigt Employee of: CSL Behring, I. Jacobs Employee of: CSL Behring, E. Santagostino Grant/Research Support from: Pfizer, Consultant for: CSL Behring, Grifols, Bayer, Baxter, Speaker Bureau of: Kedrion, Octapharma, Biotest.

OR347

Efficacy and safety results of a phase 3 pivotal clinical study of recombinant fusion protein linking coagulation factor IX with albumin (RIX-FP) in previously treated patients with hemophilia B

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Background: A fusion protein genetically linking recombinant human coagulation FIX with recombinant human albumin (rIX-FP) has been developed with an improved PK profile, thus improving hemophilia B treatment by allowing less frequent dosing.

Aims: In the PROLONG-9FP program, safety and efficacy of rIX-FP were evaluated for prophylaxis treatment (PT) of every 7-, 10- and 14-days and on-demand treatment (ODT) of bleeding episodes in previ-

ously treated male patients (12–61 years) with hemophilia B (FIX $\leq 2\%$) for 12–18 months.

Methods: Subjects in the on-demand arm received only ODT for 6 months and then switched to 7-day PT. Subjects in the prophylaxis arm received 7-day PT for 6 months, and eligible subjects switched to 10- or 14-day PT interval. Annualized spontaneous bleeding rates (AsBR) were compared between ODT and PT periods (on-demand arm), and between 7-day PT and 10- or 14-day PT (prophylaxis arm). Treatment efficacy was evaluated by the number of injections to achieve hemostasis.

Results: A total of 63 subjects were enrolled from Europe, Japan, Israel and US. In the on-demand arm, 19/23 subjects switched to 7-day PT after completing 6 months ODT. The median (Q1, Q3) AsBR during ODT and PT was 15.43 (7.98, 17.96) and 0.00 (0.00, 0.96), respectively, a reduction of 100% ($P < 0.0001$). Twenty-one subjects extended their treatment interval to 14-day PT. All PT subjects ($n = 40$) on 7-, 10- and 14-day PT had a median AsBR of 0.00. Subjects on 14-day PT ($50\text{--}75 \text{ IU kg}^{-1}$) reduced consumption by 50% over their prior FIX product. A total of 98.6% of bleeding episodes were successfully treated with ≤ 2 injections of rIX-FP, 93.6% with 1 injection. No subjects developed inhibitors to FIX or antibodies to rIX-FP and there were no related serious adverse events during the study.

Conclusion: This phase 3 study demonstrated the clinical efficacy of rIX-FP for routine prophylaxis once 7-, 10- and 14-days and on-demand treatment of bleeding episodes. In addition, rIX-FP demonstrated favorable long-term safety and tolerability.

Disclosure of Interest: E. Santagostino Grant/Research Support from: Pfizer, Consultant for: CSL Behring, Grifols, Bayer, Baxter, Speaker Bureau of: Kedrion, Octapharma, Biotest, U. Martinowitz: None declared, T. Lissitchkov: None declared, B. Pan-Petes: None declared, H. Hanabusa: None declared, J. Oldenburg Grant/Research Support from: Baxter, Bayer, Biotest, CSL Behring, Grifols, Novo Nordisk, Octapharma, Swedish Orphan Biovitrum, Pfizer, Consultant for: Baxter, Bayer, Biogen Idec, Biotest, CSL Behring, Grifols, Novo Nordisk, Octapharma, Swedish Orphan Biovitrum, Pfizer, Speaker Bureau of: Baxter, Bayer, Biogen Idec, Biotest, CSL Behring, Grifols, Novo Nordisk, Octapharma, Swedish Orphan Biovitrum, Pfizer, L. Boggio: None declared, C. Negrier Consultant for: CSL Behring, I. Pabinger-Fasching Grant/Research Support from: CSL Behring, Consultant for: CSL Behring, M. von Depka Prondzinski: None declared, C. Altisent: None declared, G. Castaman Consultant for: CSL Behring, K. Yamamoto: None declared, M. Álvarez-Román Consultant for: Baxter, Bayer, NovoNordisk, Pfizer, C. Voigt Employee of: CSL Behring, I. Jacobs Employee of: CSL Behring.

OR348

Perioperative hemostatic management of major surgery in hemophilia B with long-acting recombinant glycopegylated factor IX: results from the paradigmTM3 clinical trial

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Background: Current challenges in hemophilia B patients undergoing major surgery are the need for frequent monitoring of factor IX (FIX) levels and the risk of postoperative bleeding. Nonacog beta pegol, a recombinant glycoPEGylated FIX with a prolonged half-life offering high trough levels (Blood 2011;118:2695), has the potential to improve perioperative management of hemophilia B patients.

Aims: To evaluate efficacy and safety of nonacog beta pegol in hemophilia B patients undergoing major surgical procedures.

Methods: A surgery trial was conducted in 13 previously treated hemophilia B patients (FIX $\leq 2\%$). The trial was approved by independent ethics committees and patients provided written informed consent. All patients received a preoperative bolus injection of 80 IU kg⁻¹. Postoperatively, the patients received a fixed dose of 40 IU kg⁻¹ repeated at the investigator's discretion. Efficacy was assessed intra- and postoperatively. Safety assessments included monitoring of adverse events and immunogenicity.

Results: Apart from the preoperative injection, none of the patients needed additional doses on the day of surgery. The median (range) number of postoperative doses was 2.0 (0–4) from Day 1 to 6 and 1.5 (0–3) from Day 7 to 13, which compares well with previously modelled PK results (J Thromb Haemost 2012;10:2305). The mean total consumption was 241 IU kg⁻¹ from Day of surgery to Day 14+. Intraoperative hemostatic effect was excellent/good in all 13 cases. Based on the type and extent of the surgeries, no unexpected intra- or postoperative bleeding or other complications were observed. No patients developed inhibitors. No thromboembolic events were observed.

Conclusion: The results indicate that nonacog beta pegol was safe and effective for surgery. Perioperative consumption and number of injections were considerably lower than reported for any other FIX products offering distinct advantages over currently used frequent bolus injections and continuous infusion.

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gen and CSL Behring, Paid Instructor at: Bayer, Biogen and CSL Behring.

OR349

Improved health-related quality of life (QoL) of haemophilia B patients treated with a weekly 40 IU kg⁻¹ dose of a new long-acting recombinant factor IX (FIX) product, nonacog beta pegol

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Background: Haemophilia B is an X-linked recessive disorder in which bleeding can occur in joints, muscles and soft tissues, potentially affecting patients' QoL. Management includes injection of FIX 2–3 times/week to prevent bleeds [prophylaxis (PPX)] or as on-demand treatment (OD) of bleeds. Nonacog beta pegol is a long-acting FIX product enabling less frequent injections.

Aims: To assess QoL of haemophilia B patients treated with nonacog beta pegol.

Methods: Patients aged 13–70 years with FIX activity level $\leq 2\%$ were included in a single-blind, multi-national phase 3 trial evaluating safety and clinical efficacy of nonacog beta pegol. Patients were treated OD for 28 weeks or randomised to once weekly PPX (10 IU kg⁻¹ or 40 IU kg⁻¹) for 52 weeks. Questionnaires were completed at baseline (BL) and at end-of-trial (EOT) visit. Adults (17–70 years) completed the Haemophilia-Adults-Quality of life (HAEM-A-QOL) questionnaire. For children (13–16 years), parent and children versions of the Haemophilia-Quality of life questionnaire (HAEMO-QOL) were completed. Changes from BL to EOT visit in each treatment arm were tested using the Wilcoxon signed rank test.

Results: There were 59 adults (13 in OD, 24 in PPX 10 IU kg⁻¹ and 22 in PPX 40 IU kg⁻¹) and 15 children (2 in OD, 6 in PPX 10 IU kg⁻¹ and 7 in PPX 40 IU kg⁻¹) in the trial, mean age 33 and 14 years respectively. The PPX 40 IU kg⁻¹ adult arm reported significant improvements (negative change) for the HAEM-A-QOL domains: Feeling (-15.2 ± 18.3 , $P = 0.0098$), Sport (-15.3 ± 19.0 , $P = 0.0195$), Partnership (-9.6 ± 15.5 , $P = 0.0313$) and total HAEM-A-QOL score (-6.4 ± 8.5 , $P = 0.0166$). No significant changes were reported in the OD or PPX 10 IU kg⁻¹ adult arm, or for children in any treatment arm.

Conclusion: Adults treated with 40 IU kg⁻¹ nonacog beta pegol once weekly PPX reported significant improvements in several key HAEM-A-QOL domain scores and in the overall HAEM-A-QOL score. For adults on the 10 IU kg⁻¹ week⁻¹ PPX or OD and for children, QoL was unchanged.

Disclosure of Interest: P. Chowdary Grant/Research Support from: Novo Nordisk, Pfizer, CSL Behring, Consultant for: Novo Nordisk, Pfizer, CSL Behring, Baxter, Biogen Idec, Speaker Bureau of: Bayer, S. Kearney Grant/Research Support from: Local PI for Novo Nordisk, Bayer and Biogen Studies, Consultant for: Biogen Ad Board Participant, D. Yee: None declared, J. Meunier Employee of: Mapi, U. Slothuus Employee of: Novo Nordisk, C. Hoxer Employee of: Novo Nordisk.

OR350

Population pharmacokinetics (PK) of recombinant fusion protein linking coagulation factor IX with recombinant albumin (RIX-FP) in adult and pediatric patients with severe hemophilia B

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Background: rIX-FP was developed to extend the half-life of FIX, improving hemophilia B treatment by allowing less frequent dosing than the standard FIX products. Four trials in the PROLONG-9FP clinical program were completed in previously treated adult and pediatric patients with hemophilia B (FIX $\leq 2\%$). To our knowledge, no population PK (PPK) model has been undertaken for FIX products to simultaneously analyze FIX activity data from patients aged 1–61 years (y).

Aims: A PPK model was developed to characterize rIX-FP PK, to describe and identify demographic and clinical covariates of rIX-FP PK variability and to simulate FIX activity-time profiles for various dosing regimens.

Methods: Blood PK samples from 104 patients were collected to determine the plasma FIX activity using a validated one-stage clotting assay. PPK modeling was performed using NONMEM 7, including the assessment of potential covariates on rIX-FP PK. Visual predictive check (VPC) was used for model evaluation.

Results: A 2-compartmental model appropriately described the rIX-FP PK. Body weight was a significant covariate on clearance and both central and peripheral volumes of distribution, and weight-adjusted dose was a significant covariate on central volume. The VPC results confirmed model stability, and the PK parameters were estimated with good precision. For the respective age groups of ≥ 12 years, 6 to < 12 years and < 6 years, simulations based on the final PPK model predicted a median trough activity of 5%, 2% and 1% after 50 IU kg⁻¹ rIX-FP once every 14 days, and 8, 4, 3% after 25 IU kg⁻¹ once weekly. Time to 1% after a single dose was also estimated.

Conclusion: The PPK model adequately characterized rIX-FP PK. The PK parameters estimated by the PPK model were consistent with those by the non-compartmental approach, and results of the prediction support FIX activity measured in clinical studies. This model can be utilized as a tool to simulate FIX activity-time profiles for various dosing scenarios of rIX-FP.

Disclosure of Interest: T. Limsakun Employee of: CSL Behring, Y. Zhang Employee of: CSL Behring, J. Sidhu Employee of: CSL, D. Bensen-Kennedy Employee of: CSL Behring, A. Feussner Employee of: CSL Behring GmbH, C. Voigt Employee of: CSL Behring, I. Jacobs Employee of: CSL Behring, E. Santagostino Consultant for: CSL Behring, M. Massimo Consultant for: CSL Behring.

Contact System II

OR351

Role of FXIIa, FXIa, and platelet-derived polyphosphate in thrombin generation using human whole blood perfused over a collagen/tissue factor surface

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Background: FXII and FXI contribute to thrombosis in animal models. Platelet derived polyphosphate serve as FXII activator and mediator for thrombin activation of FXI, while attenuating fibrinolysis by enhancing fibrin structure. However, these pathways have not been well quantified with human blood under defined flow conditions over defined surfaces.

Aims: To develop a microfluidic assay of platelet deposition and coagulation on collagen/tissue factor (TF) surfaces that can trigger thrombin generation via contact and/or extrinsic pathway and to differentiate the relative contribution of FXIIa, FXIa, and polyphosphate in clot formation, stability, and function under flow.

Methods: Human blood was anticoagulated with low [CTI] (corn trypsin inhibitor, 4 $\mu\text{g mL}^{-1}$) and was labeled with fluorescent anti-CD 61 antibody and fluorescent fibrinogen. Blood was treated with FXI antibody 14E11 (blocks FXI activation by FXIIa), O1A6 (blocks FIX activation by FXIa) or PPXbd (polyphosphate binding protein) and perfused over collagen/TF surface at 100 s⁻¹ in 8-channel microfluidic device within 5 min of phlebotomy. Platelet deposition and fibrin formation were monitored by fluorescent microscopy for 400 s (or 1600s if tPA was added to study fibrinolysis).

Results: 14E11 and O1A6 blocked fibrin formation under null TF condition. At low TF level ($< 0.1 \text{ molec } \mu\text{m}^{-2}$), both antibodies reduced fibrin formation. In contrast, at high TF level ($> 0.5 \text{ molec } \mu\text{m}^{-2}$), fibrin formation was normal. Inhibiting polyphosphate only delayed fibrin formation at low TF level. Thrombi formed with PPXbd were more susceptible to lysis and experienced more contraction. Platelet deposition was not affected by the two antibodies or PPXbd.

Conclusion: Contact pathway was indispensable for thrombin generation under null TF condition. Polyphosphate modulates clotting under flow primarily through: (i) potentiating thrombin activation of FXI, (ii) enhancing fibrin structure, (iii) attenuating contraction.

Disclosure of Interest: None declared.

OR352

Antithrombotic effect of ONO-8610539, a new, potent and selective small molecule factor XIa inhibitor, in a monkey model of arteriovenous shunt

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Background: Patients with severe factor XI (FXI) deficiency have a low risk of deep vein thrombosis. In addition, FXI antisense oligonucleotide prevented venous thrombosis in human and seems to be safe in terms of bleeding risk (N Engl J Med 2015;372:232-240). Thus, FXI is considered to be a promising drug target.

Aims: ONO-8610539 is a new, potent and highly selective small molecule inhibitor of activated human FXI (FXIa), and inhibited venous thrombosis in rabbits without increasing bleeding risk [Gohda et al.

Blood 2014;124(21):1542]. We evaluated *in vitro* anticoagulant and *in vivo* antithrombotic effects of ONO-8610539 in monkeys.

Methods: Activated partial thromboplastin time (APTT) and prothrombin time (PT) were measured *in vitro*, and the APTT-doubling concentration (APTT2) was calculated. The saphenous artery and vein of the hind leg of cynomolgus monkey were connected by an arteriovenous (AV) shunt in which a copper wire was placed. Blood was perfused to the AV shunt for 15 min to form a thrombus, followed by measurement of thrombus weight on the wire. ONO-8610539 intravenous infusion was started 60 min before blood perfusion. Blood samples were collected just before blood perfusion to measure plasma ONO-8610539 concentration, APTT, and PT.

Results: ONO-8610539 showed an anticoagulant effect in monkey plasma with APTT2 of 130 nmol L⁻¹, which is almost equal to human APTT2 (98 nmol L⁻¹), however, it barely prolonged PT at 33 μmol L⁻¹. ONO-8610539 significantly inhibited thrombus formation in monkeys at doses of 6 μg kg⁻¹ h⁻¹ or higher. ONO-8610539 at 6 μg kg⁻¹ h⁻¹ produced a plasma concentration of 39.4 nmol L⁻¹ and 1.4-fold APTT prolongation over control. We are assessing the hemorrhagic effect in monkeys and will show the result at the presentation.

Conclusion: ONO-8610539, a new, potent and selective small molecule FXIa inhibitor, showed potent anticoagulant and antithrombotic effects in monkeys, hence is expected to be a promising anticoagulant drug without increasing bleeding risk.

Disclosure of Interest: None declared.

OR353

Platelet polyphosphate accelerates the inhibition of TFPI by factor XIa

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Background: Alpha tissue factor pathway inhibitor (TFPIα) can be neutralized by activated factor XI (FXIa), accelerating plasma clotting time and fibrin generation, although this reaction is slow and requires relatively high concentrations of FXIa. Activated platelets secrete polyphosphate (polyP), which accelerates the activation of FXI by thrombin and the activation of FV by FXIa. PolyP is also able to inhibit the anticoagulant effect of TFPIα in plasma but the mechanism for this reaction is still uncertain.

Aims: The aim of the present study was to determine whether platelet polyP is a cofactor for the inhibition of TFPIα by FXIa.

Methods: To measure the binding of polyP to TFPI, biotinylated-polyP was added to TFPIα-coated wells. Binding was detected with HRP-streptavidin. Activated FX (FXa) inhibition by TFPIα was measured in the FXa-initiated clotting time of FX-depleted plasma. TFPIα inhibition of FXa generation by the tissue factor (TF)-FVIIa complex was measuring using a FXa chromogenic substrate.

Results: We found that polyP was able to bind to TFPIα in a concentration-dependent manner. In the absence of polyP, the pretreatment of TFPIα (5 nM) with 1 nM FXIa for 1 h abrogated the anticoagulant effect of TFPIα in the FXa-initiated clotting time of FX-depleted plasma. In the presence of polyP (10 μM), the pretreatment of TFPIα (5 nM) with 0.25 nM FXIa for 30 min was enough to completely abrogate the anticoagulant effect of TFPIα in plasma. Also, the presence of polyP potentially accelerated the effect of FXIa to inhibit the capacity of TFPIα to block the generation of FX by the TF-FVIIa complex.

Conclusion: Our study provides a novel molecular link between hemostatic activation of platelets and FXI. The results suggest that the hemostatic role of FXIa may be attributed not only to activation of FIX

but also to promoting the extrinsic pathway of thrombin generation through inactivation of TFPIα by FXIa, which is significantly enhanced by platelet-derived polyP.

Disclosure of Interest: None declared.

OR354

Binding of polyphosphate to contact pathway enzymes is dependent on transition metals

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Background: Inorganic polyphosphates (polyP) are linear phosphate polymers that are secreted from platelet dense granules and modulate blood clotting and inflammation. We previously reported that polyP enhances autoactivation of factor (F) FXII and FXI, activation of FXI by thrombin, and activation of FV by FXIa. Zinc ions have been reported to contribute to the activities of enzymes and cofactors in the contact pathway of blood clotting. PolyP is known to bind to many divalent metal ions, but the role of transition metals bound to polyP in blood clotting is not understood. In particular, it is likely that previous studies from our lab and others of the interactions of polyP with blood clotting proteins included unknown amounts of various trace metal ions already bound to polyP.

Aims: Evaluate the role of transition metal ions in the interaction of polyP with FXIIa, FXIa and kallikrein.

Methods: FXIIa, FXIa, and kallikrein were exhaustively depleted of trace divalent metal ions, as were buffers. Biotinylated polyP was captured on streptavidin-coated microplates, then incubated with FXIIa, FXIa, or kallikrein with 10 mM EDTA or various concentrations (0–30 μM) of CoCl₂, CuCl₂, NiCl₂ or ZnCl₂. After washing, bound enzymes were quantified using appropriate chromogenic substrates.

Results: FXIIa, FXIa and kallikrein did not bind polyP in the presence of EDTA. Binding of FXIIa to polyP was greatly stimulated by 1 μM Co²⁺, Cu²⁺, Ni²⁺ or Zn²⁺. FXIa binding to polyP was greatly stimulated by 5 μM Cu²⁺, less so with Ni²⁺ or Zn²⁺, and not at all with Co²⁺. Kallikrein binding to polyP was minimal with Co²⁺, Cu²⁺ or Ni²⁺, but increased in a concentration-dependent manner with Zn²⁺. Binding of none of these enzymes to polyP was enhanced by Ca²⁺ or Mg²⁺.

Conclusion: Interactions between polyP and enzymes of the contact pathway are highly dependent on transition metal ions, with FXIIa, FXIa and kallikrein displaying different metal ion-dependences.

Disclosure of Interest: None declared.

OR355

Coagulation factor XII binding to apoptotic cells initiates thrombin generation

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Background: Apoptosis occurs in a variety of pathological conditions and it has been well known that apoptotic cells are procoagulant and are associated with thrombotic disorders. However, the mechanisms underlying procoagulant activity of apoptotic cells remain poorly understood. We recently found that factor XII (FXII) binds to apoptotic cells and becomes cleaved, suggesting that the activation of the contact system contributes to procoagulant activity of apoptotic cells.

Aims: In this study, we determined the role of FXII in thrombin generation mediated by apoptotic cells.

Methods: The binding of FXII to apoptotic cells and FXII activation were measured by flow cytometry, Biacore and FXIIa chromogenic assay respectively. The contribution of FXII in clotting time and thrombin generation on apoptotic cells was further characterized.

Results: (1) FXII bound to apoptotic cells in a concentration-dependent manner, PS liposome significantly inhibited the binding. The sensorgrams showed an increase in response units that was reflective of PS binding, and the binding response was concentration dependent. Incubation of FXII with apoptotic cells, but not viable cells, resulted in its rapid cleavage and activation. (2) Compared with viable cells, apoptotic cells significantly shortened clotting time of recalcified citrated plasma. However, FXII-deficient plasma exhibited markedly-prolonged clotting time mediated by apoptotic cells of normal plasma, which was reversed by the reconstitution of FXII. In a thrombin generation assay, apoptotic cells significantly increased thrombin generation, however blocking of PS by annexin V, inhibition of FXII or the deficiency of FXII diminished apoptotic cells-induced thrombin generation. Addition of 375 nM FXII to FXII-deficient plasma recovered thrombin generation near to the normal plasma level.

Conclusion: FXII binds to apoptotic cells via PS leading to thrombin generation. Our observations reveal a novel mechanism underlying apoptotic cells-mediated procoagulant activity.

Disclosure of Interest: None declared.

Cancer and thrombosis – basic – I

OR356

Synergistic anti-tumor effects with dabigatran etexilate and cisplatin in a murine ovarian cancer model

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Background: Cancer is often associated with an increased risk of thrombotic events which can be exacerbated by treatment with standard chemotherapeutic agents. Both coagulation factors and thrombin are increased in tumors. Thrombin can drive cancer progression directly through the activation of protease-activated receptors and platelets or indirectly by generating fibrin matrices.

Aims: To investigate the extent and the mechanism by which treatment with the oral thrombin inhibitor, dabigatran etexilate (DE), acts synergistically with cisplatin to block tumor spread in a murine ovarian tumor model.

Methods: The effect of combined treatment with cisplatin and DE was evaluated following i.p. injection of ID8 ovarian adenocarcinoma cells in C57BL/6 mice. Peritoneal tumor spread of ID8 tumor cells expressing the luciferase reporter gene was monitored by bioluminescence imaging. Treatment was initiated when ID8 bioluminescence was elevated at 5 weeks post ID8 tumor cell injection. Mice were treated once a week with low dose cisplatin (1 or 2 mg kg⁻¹, i.p.) with or without DE (oral gavage, 80 mg kg⁻¹, bid or supplemented in the chow).

Results: Although low dose cisplatin alone or DE alone did not inhibit peritoneal tumor spread, co-treatment with both DE and cisplatin significantly ($P \leq 0.05$) decreased ID8 tumor spread as measured by bioluminescence. The number of circulating tissue factor⁺ microparticles (TF⁺ MPs) (particularly of MPs of platelet origin) in DE-treated tumor-bearing mice was decreased to levels found in non-tumor bearing mice. Analyses of ascites fluid collected from tumor-bearing mice revealed that levels of cytokines (including IL-6, MCP-1, IL-10, and

TGF-β) were decreased by ~75% in mice treated with DE with or without cisplatin.

Conclusion: These results suggest that dabigatran may be beneficial in not only preventing thrombotic events in cancer patients, but also as adjunct therapy to treat malignant ovarian tumors. (Supported by funds from Boehringer Ingelheim Pharma).

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OR357

Podoplanin expression and intravascular platelet aggregates: the missing link between cancer and thrombosis in primary malignant brain tumors

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Background: Venous thromboembolism (VTE) is a common clinical problem in patients with primary malignant brain tumors, and underlying mechanisms are unclear.

Aims: We explored the association of podoplanin, a sialomucin-like glycoprotein that has the ability to induce blood platelet activation and aggregation, with VTE in primary malignant brain tumors.

Methods: Immunohistochemical staining against podoplanin and platelet surface protein CD61 was performed in primary brain tumor specimens of 213 adult patients (mostly high-grade gliomas [89%]) included in the Vienna Cancer and Thrombosis Study (CATS), a prospective observational cohort study of patients with newly diagnosed cancer or progressive disease. Primary endpoint was symptomatic VTE.

Results: During 2-year-follow-up, 29 (13.6%) patients developed VTE. In total, 151 (70.9%) tumor specimens stained positive for podoplanin (33 high expression, 47 medium expression, 71 low expression). Patients with podoplanin positive tumors had a lower blood platelet count (Median [25th–75th percentile], G/I: 227 [186–285] vs. 286 [241–355]; $P < 0.001$) and higher D-dimer levels (mg mL⁻¹: 0.85 [0.46–1.92] vs. 0.42 [0.23–0.79]; $P < 0.001$). Increasing podoplanin staining intensity was associated with increasing levels of CD61-positive intravascular platelet aggregates in tumor specimens ($P < 0.001$).

In Cox regression analysis, high podoplanin expression was associated with an increased risk of VTE (hazard ratio [HR] for high vs. no podoplanin expression: 5.75, 95% confidence interval [CI]: 1.71–19.27; $P = 0.005$). This association was independent of age, sex and tumor grade (HR 5.71, 95% CI: 1.52–21.26; $P = 0.010$).

Conclusion: High podoplanin expression in primary malignant brain tumors, which correlates with intravascular platelet aggregates, lower blood platelet counts and hypercoagulability, is associated with increased risk of VTE. Our study might provide a novel mechanistic insight into the pathogenesis of VTE in patients with primary malignant brain tumors.

Disclosure of Interest: None declared.

OR358

The gene encoding vitamine K-dependent anticoagulant protein S: a positive regulator of lung cancer cell migration and invasion

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Background: Cancer is often associated with venous thrombosis, a phenomenon that was described by Trousseau. However the molecular basis of this interaction is not well understood. Protein S (PS) is now emerging as a key anticoagulant protein at the crossroads of multiple biological processes. Following binding to a unique family of protein tyrosine kinase receptors referred to as Tyro-3, Axl and Mer (TAM) receptors, PS can lead to regulation of coagulation, phagocytosis of apoptotic cells, cell survival, activation of innate immunity, angiogenesis, and cancer progression.

Aims: The aim of this study is to address the role of PS in cancer development and progression.

Methods: We used adenoviral overexpression of PS and generated stable knock-down cell lines expressing shRNA targeting PS.

Results: We now demonstrate that PS is overexpressed in clinical lung tumor samples. Using a panel of lung carcinoma cell lines derived from tumors of all major histologic subtypes, we also showed that PS is significantly upregulated in all cell lines compared to normal human bronchial epithelial cells (NHBE) or nontumorigenic human bronchial epithelial cell line derived from normal bronchus. Regulating PS expression using either a replication-incompetent adenovirus or PS shRNA lentiviral particles expressing sense PS or antisense PS, respectively, modifies the transformed phenotypes (i.e. migration and invasion) and alters colony formation and tumor growth ability *in vivo*. A direct relationship is observed between PS expression and increased phosphorylation between of ERK1/2, JNK, and p38/MAPK pathways. Treatment of lung cancer cells with TAM receptors shRNA lentiviral particles decreased Tyro-3 and Mer-induced PS signaling pathways.

Conclusion: The present finding uncover a new role of PS as an important anticoagulant protein that initiates through TAM receptors multiple signaling pathways and leads to cancer progression. PS may provide a promising new focus to the management of thrombotic complications associated with cancer.

Disclosure of Interest: None declared.

OR359

Anti-tumor role of antithrombin by inhibition of enteropeptidase through a novel mechanism

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Background: Antithrombin (AT) is a key inhibitor of the coagulation cascade, whose deficiency increases the risk of thrombosis. However, AT may also function as anti-inflammatory, anti-viral and anti-apoptotic.

Aims: To explore the potential anti-tumor effect of AT by characterizing its ability to inhibit a protease involved in metastasis, enteropeptidase (EP).

Methods: Characterization (electrophoretic and chromogenic assays) of the interaction between EP and AT using purified proteins and AT mutants (R47C and DelR393), and using cells expressing EP on their

surface (LoVo, SK-BR-3 and U-87MG). Angiogenesis of endothelial cells, experiments of wound healing and degradation of a gelatin matrix mediated by invadopodia using U-87MG cells. Immunohisto-fluorescence of EP and AT in tumors surgically removed from carcinoma cell xenografts in *nu/nu* mice by confocal microscopy.

Results: AT was able to inhibit EP *in vitro* and on the cells membrane surface. This process required the activation of AT by heparin, being low molecular heparin the most efficient cofactor in comparison with pentasaccharide and unfractionated heparin. Characterization of this inhibition revealed that both functional domains of AT, the reactive centre loop and the heparin binding site, are required. Interestingly, the mechanism of inhibition of EP by AT is slightly different than that used on procoagulant proteases, as it exerts a non-covalent inhibition of this target. The addition of AT-heparin to U-87MG cells reduced cell migration and invasion. Finally, carcinoma cell xenografts in nude mice cells revealed the co-localization of EP and AT *in vivo*. Moreover, as a consequence of the EP inhibition, AT is cleaved and in such conformation has anti-angiogenic effects reducing the vessel formation of endothelial cells.

Conclusion: AT inhibits EP by a novel mechanism that may have a double anti-tumor effect, as a protease involved in metastasis is inhibited and an anti-angiogenic molecule is generated.

Disclosure of Interest: None declared.

OR360

Expression of coagulation proteases from the APC pathway in tumours from gynaecological cancer patients with and without venous thrombosis

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Background: Gynaecological malignancies have been associated with high rates of venous thromboembolism (VTE). Thrombus formation is complex in cancer and may be explained by release of procoagulant material from malignant tumours.

Aims: The aim of this study was to compare gene expression of proteases of the APC pathway in tumours from gynaecological cancer patients with and without VTE complications compared with benign tumours.

Methods: RNA was extracted from 78 stored fresh frozen tumour samples. All patients gave informed consent and the study was approved by the local ethics committee. 27 cases of gynaecological cancer complicated by VTE were identified (ovarian *n* = 19, endometrial *n* = 8) and matched to 27 malignant cases without VTE (ovarian *n* = 20; endometrial *n* = 7) and compared with 24 benign cases. mRNA expression of endothelial protein C receptor (EPCR), protein S (PS), protein C (PC), thrombomodulin (TM), Factor V (FV) and VIII (FVIII) was measured using TaqMan Low Density Arrays.

Results: When compared with benign tumours, mRNA expression of EPCR (*P* < 0.004; *P* < 0.02), PS (*P* < 0.001; *P* < 0.001) and FVIII (*P* < 0.013; *P* < 0.004) were significantly down-regulated and FV was significantly up-regulated (*P* < 0.001; *P* < 0.002), in tumours from patients with malignancies complicated by VTE and malignancies who were VTE free respectively. TM was downregulated in malignant tumours from both groups but this did not reach statistical significance. Low levels of PC expression was found in some tumours but this was inconsistent across all tissues. There was no significant differences in mRNA expression from patients with gynaecological malignancies complicated by VTE compared with those who were VTE free.

Conclusion: Changes in tumour mRNA expression of proteins in the APC pathway does not explain the VTE associated with gynaecological malignancy. Reduced expression of key proteins associated with activation of protein C combined with increased expression in FV may contribute to local thrombin production and tumour progression.

Disclosure of Interest: None declared.

ADAMTS 13

OR361

Exosite interactions uncover a broad substrate recognition profile for ADAMTS13

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Background: The metalloprotease ADAMTS13 (Ats13) cleaves von Willebrand Factor (VWF), reducing multimer length and controlling platelet binding activity. Ats13 is secreted as an active protease, has no known natural inhibitors, and VWF is its only known substrate. These unique features suggest that Ats13 exhibits a narrow substrate specificity profile.

Aims: Define the breadth of Ats13 substrate specificity.

Methods: A substrate phage display library (6N) consisting of random 6 amino acid (AA) peptides inserted between the PIII protein and a FLAG tag was screened by proteolysis with Ats13 or thrombin (IIa) to define their substrate sequence requirements. A second library (6N-VWF73) was also constructed with random 6 AA peptides substituted for the P3-P3' residues in VWF73 (a 73 AA fragment of VWF). Cleaved phages were recovered and the sequences determined using high throughput DNA sequencing (HTS).

Results: The 6N library (2.3×10^8 independent clones) accounted for all 6.4×10^7 possible AA combinations at the 6 positions. Selection by IIa revealed 6722 cleaved peptides, 99.7% of which contained Arg. As expected, P-R-S was the dominant motif, validating our method. Selection of the 6N library by Ats13 yielded 96 cleaved peptides. V, W, M, and L were enriched among cleaved peptides, but no clear motif emerged. By contrast, Ats13 cleaved 1670 unique peptides from 6N-VWF73. L-V-Y-M-V-T (the native P3-P3' sequence in VWF) was among the top 100 enriched peptides. ~75% of peptides contained L, with 568/1670 containing L at P3. Analysis of depleted peptides (representing consistently uncleaved peptides) revealed enrichment of E, D, and P, suggesting that these AA discouraged Ats13 substrate recognition.

Conclusion: Exosite interaction uncovers a broader substrate recognition profile for Ats13, and appears to be a strong requirement for novel substrate recognition. Ats13 exhibits strong preference for AA near the scissile bond, a feature that is unique among metalloproteases.

Disclosure of Interest: None declared.

OR362

Timely formation of ULVWF multimers and reduction of ADAMTS13 activity precede clinical events of TTP relapse

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Background: VWF function and ADAMTS13 (AD13) activity are critically involved in TTP's pathogenesis but the time-course relationship

between reduction of AD13 activity, formation of ULVWF, and clinical event of TTP relapse is unclear.

Aims: Studying the biomarkers predictive of TTP relapse.

Methods: The OSU-TTP program has been following all TTP patients throughout clinical remission with samples collected quarterly up until the event of relapse. VWF multimer and AD13 activity were studied in 97 quarterly samples, including 43 pre-relapse (PR) samples that were collected within 3 months prior to TTP relapse, 33 samples from relapsers (R) that were collected in the quarters without a relapse in the subsequent 3 months, and 21 controls from nonrelapsers.

Results: The normal range of ULVWF was defined by 40 donors. ULVWF was present in 74% of PR samples, 52% of R group, and 14% of NR controls. When ULVWF was quantified as a % of the total VWF protein in the sample, median ULVWF levels were significantly higher in both PR (7.8%, $P < 0.05$) and R (5.9%, $P < 0.05$) groups than NR controls (1.6%). In contrast, AD13 activities were significantly lower in both PR (5%, $P < 0.05$) and R (19%, $P < 0.05$) groups than NR group (70%). Overall, the increase of ULVWF density was correlated with a reduction in AD13 in these 97 quarterly samples ($r = -0.46$, $P < 0.05$). For patients that showed a $< 20\%$ AD13 activity, ROC analysis indicated that a presence of ULVWF in PR samples would predict a relapse in $> 60\%$ of cases. When the data was analyzed as a time-course to relapse (i.e. 8–12 weeks, 4–8 weeks, 2–4 weeks, and < 2 weeks), the PR samples showed an increasing trend in ULVWF toward the time point of relapse, with ULVWF present in $> 90\%$ of the samples collected within < 2 weeks prior to relapse.

Conclusion: Patients with a history of relapse exhibit a baseline increase in ULVWF and a decrease in AD13 throughout remission. A substantial increase in ULVWF along with a large reduction of AD13 is a significant risk factor predicting TTP relapse.

Disclosure of Interest: None declared.

OR363

The predictive value of ADAMTS13 activity for treatment monitoring of patients with acquired TTP: data from the phase II TITAN trial with caplacizumab

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Background: ADAMTS13 activity (ATS13) is increasingly used to guide therapy in patients with acquired thrombotic thrombocytopenic purpura (TTP). Results from the phase II TITAN trial demonstrated that the anti-von Willebrand Factor Nanobody, Caplacizumab (CAP) significantly reduced the incidence of exacerbations. In some patients relapses occurred shortly after CAP stop, suggesting that underlying disease activity was not yet resolved and that longer CAP treatment could maintain the treatment benefit in these patients and prevent these relapses.

Aims: The predictive value of ATS13 as marker of underlying disease activity and for exacerbation or relapse was evaluated, using prospective data from the TITAN trial.

Methods: ATS13 was measured by the FRET-VWF73 assay. Subjects with missing data or ATS13 $\geq 10\%$ at screening were excluded from the analysis. ATS13 $< 10\%$ was considered as indicative for active disease.

Results: ATS13 was $< 10\%$ at screening in 77% of patients in the placebo (PLC) arm and 78% of CAP subjects (8% and 17% missing), respectively. The prognostic value of ATS13 was shown in the PLC arm: 94% of subjects without exacerbation or relapse showed a recovery of ATS13 $\geq 10\%$ one week post daily plasma exchange (PE). Conversely, ATS13 was $< 10\%$ in 88% of PLC subjects around the time of exacerbation.

Similar trends were observed in the CAP arm: ATSI3 was $\geq 10\%$ near the end of the treatment period in 81% of CAP subjects without exacerbation or relapse. Seven subjects had a relapse within 4–10 days of stopping CAP; all had continuous ATSI3 $< 10\%$ during CAP treatment. Four subjects with relapses occurring ≥ 30 days after stopping CAP had ATSI3 values $\geq 10\%$ near treatment stop, but ATSI3 was again $< 10\%$ around the time of relapse.

Conclusion: Our data highlight that ATSI3 identifies acquired TTP patients at risk for exacerbation or relapse and may be considered as guide to extend duration of caplacizumab treatment for the prevention of recurrences of the presenting TTP episode.

Disclosure of Interest: F. Callewaert Employee of: Ablynx NV, H. Ulrichs Employee of: Ablynx NV, J. A. Kremer Hovinga Paid Instructor at: Investigator during Phase II TITAN study; central lab analysis of ADAMTS13 activity, K. De Swert Employee of: Ablynx NV, D. Tersago Employee of: Ablynx NV.

OR364

Low ADAMTS13 activity and the risk of coronary heart disease: a prospective cohort study – the Rotterdam Study

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Background: The metalloprotease ADAMTS13 cleaves high molecular weight von Willebrand factors (VWF) multimers into smaller, less procoagulant forms. Lower ADAMTS13 activity is associated with an increased risk of ischemic stroke, but whether individuals with low ADAMTS13 activity also have a higher risk of coronary heart disease (CHD) is unknown.

Aims: To determine the association between ADAMTS13 activity and the risk of coronary heart disease in a large prospective population-based cohort study.

Methods: Five thousand six hundred and eighty-eight participants of the Rotterdam Study, a population-based cohort study among individuals ≥ 55 years without a history of CHD were included. ADAMTS13 activity was measured by the FRET-VWF73 assay, and VWF:Ag levels by ELISA. We assessed the association between ADAMTS13 activity, VWF:Ag levels and coronary heart disease by Cox proportional hazard regression analysis. Informed consent was obtained and the study was approved by a recognised medical ethics committee.

Results: Over a median follow-up time of 9.7 years, 453 individuals suffered from a coronary event. Individuals with the lowest ADAMTS13 activity had a higher risk of CHD than those in the reference highest quartile (HR 1.42, 95% CI 1.07–1.89). Models that include both ADAMTS13 activity and VWF:Ag levels suggests a stronger role for ADAMTS13 than for VWF in CHD risk prediction.

Conclusion: This first large prospective cohort study shows that low ADAMTS13 activity is associated with an increased risk of coronary heart disease in the elderly, independently of established cardiovascular risk factors.

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OR365

Evolutionary variation in the allosteric properties of ADAMTS13

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Background: Human ADAMTS13 is allosterically activated by low pH, monoclonal antibodies (MAbs) against distal T-CUB domains, or binding to VWF. Animals have been used extensively in studies of thrombotic thrombocytopenic purpura, but whether their endogenous ADAMTS13 behaves similarly is not known.

Aims: Determine whether ADAMTS13 is allosterically regulated in other vertebrates.

Methods: Plasma ADAMTS13 was assayed with FRET-rVWF71 without or with human VWF D4 or MAbs 7C4 (anti T67), 19H4 (anti T8), and 12D4 (anti CUBs).

Results: At physiological pH, plasma ADAMTS13 activity was $< 0.5\%$ (guinea pig), $\sim 1.9\%$ (rat), $\sim 10\%$ (marmoset, horse, FVB mouse), $\sim 25\%$ (baboon, pig, C57Bl/6 mouse), $\sim 60\%$ (*X. laevis*), $\sim 100\%$ (macaque), $\sim 300\%$ (rabbit) and $\sim 400\%$ (dog) compared to human. Rat and guinea pig plasma also had $\sim 1.9\%$ and $\sim 1\%$ activity towards a rat variant of FRET-rVWF71, indicating that human VWF sequence did not markedly impair cleavage. Except for guinea pig, frog, dog and rabbit, ADAMTS13 from all species showed robust 3- to 18-fold activation by MAbs 7C4, 19H4 and 12D4 that activate human ADAMTS13. Human ADAMTS13 is activated $\sim 400\%$ at pH 6 but dog ADAMTS13, which lacks the T3 domain, was strikingly inhibited at pH 6 with 25% activity compared to pH 7.4. Frog and rabbit ADAMTS13 were insensitive to activation by low pH. FVB mouse full length ADAMTS13 was activated by MAbs, but C57Bl/6 mouse ADAMTS13, which is truncated after T6, was not activated by low pH or MAbs against distal T-CUB domains. Furthermore, human VWF D4 activated mouse, horse and human plasma $\sim 200\%$ to $\sim 300\%$. These results are consistent with stringent allosteric regulation of ADAMTS13 by distal T-CUB domains in most species.

Conclusion: Allosteric regulation of ADAMTS13 is broadly conserved in vertebrates, with some potential exceptions. The apparent lack of ADAMTS13 regulation in guinea pigs, rabbits, dogs, and mice with truncated ADAMTS13 needs further study but suggests they may behave differently in models of thrombotic microangiopathy.

Disclosure of Interest: None declared.

Natural anticoagulants

OR366

Development of a novel protein S-based antithrombotic agent

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Background: Protein S (PS) is a robust, negative regulator of blood coagulation whose importance is revealed dramatically by a catastrophic purpura fulminans in rare newborns homozygous for PS mutations; heterozygous individuals have an elevated risk for other serious thrombotic events.

Aims: Thrombophilia is a disorder that occurs when platelets inappropriately aggregate and/or a fibrin clot forms in an intact blood vessel or heart chamber. PS inhibits factor IXa (FIXa), the rate-limiting and sole catalyst for generation of FXa on the activated platelet surface.

Thus, we seek to use PS's FIXa inhibitory function as a preventive agent for thrombophilia.

Methods: Plasma-based clotting assays, thrombin generation assays, and isolated factor assays.

Results: As a test case, we determined whether the 8-fold increase in activity of a mutant FIXa (R338L, identified in a human patient by Simioni and co-workers) that causes thrombosis could be controlled by PS. *In vitro* (plasma and isolated factor studies) and *ex vivo* data (flow cytometry with mouse platelets) indicated that FIXa binds tightly to PS in the presence of phosphatidylserine-containing membranes and the generation of FXa by FIXa $\pm$ FVIIIa is inhibited by PS. We also found that the EGF1 domain and the heparin binding exosite region in FIXa are contact points between FIXa and PS. Finally, in an aPTT assay, the clotting activity of the proband plasma containing FIXa-R338L reverted back to the normal range with added PS. Dr. Arruda's lab maintains mice with different levels of hyperactive FIX, and we have found that addition of PS to hyperactive mouse plasmas prolongs their short aPTT clotting times.

Conclusion: PS is a potential therapy for hyperactive FIXa-induced thrombosis. We have isolated recombinant FIXa-R338L, and work is underway to characterize binding of the mutant with PS to determine an appropriate dose of PS to control hypercoagulability.

Disclosure of Interest: None declared.

OR367

Avathrin: a novel fast and tight binding competitive inhibitor of thrombin from *Amblyomma variegatum*

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Background: While attempting to feed on the host blood, ticks face the barriers of host hemostasis and host defense. To counter the host haemostatic and immune response, these blood-sucking parasites have evolved a 'magic potion' in their saliva that comprises of a diverse array of compounds with antithrombotic and immunomodulatory activities. Cardiovascular disorders are the number one cause of death globally and account for about 25% of the deaths worldwide. Therefore, there is an ever increasing demand for novel anticoagulants.

Aims: We aim to structurally and functionally characterize a novel anticoagulant, Avathrin, from the tick- *Amblyomma variegatum*. We aim to show the *in-vivo* safety and efficacy of avathrin and develop it as an anticoagulant drug.

Methods: Avathrin was synthesized by solid phase peptide synthesis and inhibition kinetics were studied using a chromogenic thrombin substrate. The structure of avathrin-thrombin complex was solved by X-ray crystallography. Thrombosis, bleeding model and pharmacokinetics experiments are being conducted in mice and rats.

Results: Avathrin is a fast tight binding competitive inhibitor of thrombin with picomolar affinity. Avathrin shows about 40% sequence identity with another thrombin inhibitor- s-variegatin. Avathrin is more than 4 orders of magnitude selective to thrombin than other plasma serine proteases. Avathrin is cleaved by thrombin and the cleaved product remains bound to the thrombin exosite and inhibits thrombin. We have solved the crystal structure of avathrin-thrombin complex and identified that the cleaved C-terminal part of avathrin binds to the thrombin exosite-I. This exosite-I binding dis-

rupts the charge relay system of the catalytic triad of the thrombin active site. Avathrin retains its inhibitory effect on thrombin for upto 36 h.

Conclusion: We have characterized avathrin, a novel thrombin inhibitor and are conducting animal experiments to demonstrate its effects *in-vivo*. We finally plan to take avathrin to the market as an anticoagulant.

Disclosure of Interest: None declared.

OR368

Increased half-life and selective function of a glycoengineered antithrombin variant

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Background: N-Glycosylation is a key post-translational modification crucial for folding, function and clearance of proteins. Thus, the glyco-engineering is a tool used to increase half life and modulate function of therapeutic proteins, which include hemostatic proteins such as FIX and APC. Antithrombin (AT), the main endogenous anticoagulant, is administered to patients with sepsis, heparin resistance, and AT-deficiency under risk conditions. Limitations of this treatment are the frequency required and potential bleedings.

Aims: To develop recombinant ATs with increased half life.

Methods: We created new N-glycosylation consensus sequences in the reactive center loop, a functional domain (G392N) and a neutral region (N73T). Expression of these variants and α and β ATs was carried out in HEK-EBNA cells. After their purification, we performed the biochemical characterization: thermal denaturation, heparin affinity and kinetics of inhibition. Proteins were radiolabeled with ¹²⁵I and kinetic clearances were evaluated upon injection in mice.

Results: Mutant ATs were efficiently glycosylated and secreted. N73T variant presented a normal function. In contrast, G392N variant did not react with FIIa in absence of heparin, and had severely affected its inhibition when activated. Surprisingly, the rates of FXa inhibition were very similar to control AT, as well as the heparin affinity. Thermal denaturation assays did not show instability of these molecules. *In vivo*, AT had a biphasic clearance. N73T mutant showed a clearance faster than controls. However, G392N variant had a slow rate of clearance at long time, leading to 2.5-fold higher levels in circulation compared to control AT at 24 h.

Conclusion: We created an AT variant with increased half-life, reduced FIIa reactivity and normal FXa inhibition as compared to AT. These properties might provide potential therapeutic advantages might reduce the frequency of administrations and bleeding complications of AT treatments.

Disclosure of Interest: None declared.

OR369

Amino acid residues in the laminin G domains of protein S involved in tissue factor pathway inhibitor interaction

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Background: Protein S functions as a cofactor for tissue factor pathway inhibitor (TFPI) and activated protein C (APC). The sex hormone binding globulin (SHBG)-like region of protein S, consisting of two laminin G-like domains (LG1 and LG2), contains the binding site for C4b-binding protein (C4BP) and TFPI. Furthermore, the LG-domains are essential for the TFPI-cofactor function and for expression of full APC-cofactor function.

Aims: The aim of the current study was to localise functionally important interaction sites in the protein S LG-domains using amino acid substitutions.

Methods: Four protein S variants were created in which clusters of surface-exposed amino acid residues within the LG-domains were substituted. Binding of protein S variants to C4BP, FVa and APC and TFPI cofactor functions were evaluated using previously established assays.

Results: All variants bound normally to C4BP, FVa and were fully functional as cofactors for APC in plasma and in pure component assays. Two variants, SHBG2 (E612A, I614A, F265A, V393A, H453A), involving residues from both LG-domains, and SHBG3 (K317A, I330A, V336A, D365A) where residues in LG1 were substituted, showed 50–60% reduction in enhancement of TFPI in FXa inhibition assays. For SHBG3 the decreased TFPI cofactor function was confirmed in plasma based thrombin generation assays. Both SHBG variants bound to TFPI with decreased affinity in surface plasmon resonance experiments. The TFPI Kunitz 3 domain is known to contain the interaction site for protein S. Using *in silico* analysis and protein docking exercises, preliminary models of the protein S SHBG/TFPI Kunitz domain 3 complex were created.

Conclusion: Based on a combination of experimental and *in silico* data we propose a binding site for TFPI on protein S, involving both LG-domains.

Disclosure of Interest: None declared.

OR370

Splicing mutations on antithrombin deficiency: diagnostic relevance and unexpected consequences

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Background: Mutations in splicing signals are increasingly recognized as responsible for human genetic disease. In antithrombin (AT) deficiency only 16 mutations affecting intron sequences (IVS) have been described (5.8% of mutations identified in *SERPINC1*, but representing up to 8% of the mutation profile of *SERPINC1*).

Aims: To characterize splicing mutations in *SERPINC1* responsible for AT deficiency.

Methods: *SERPINC1* was sequenced in 109 cases with AT deficiency. Plasma AT was studied by functional and western blot assays, purified by FPLC and characterized by proteomic analysis.

Results: We identified 12 mutations leading to aberrant splicing. P1-P8 had IVS point mutations affecting acceptor or donor sequences, 4 not previously described. P9, from a thrombophilic family, carried a 31 bp deletion affecting the final nucleotide of intron 6 and 30 bp of exon 7. P1-P9 had type I deficiency and mutations were easily detected using exon flanking primers. By contrast, P10-P11 carried more distant intronic mutations (IVS2 + 26 and IVS5-14). The IVS5-14G>A mutation was identified in a thrombophilic family with disulphide linked dimers in plasma. *In silico* prediction suggested that IVS5-14G>A creates a new acceptor sequence potentially generating a variant protein with the insertion of four residues at position 384. Purification of disulphide linked dimers from plasma of carriers and proteomic analysis confirmed the predicted splicing. Finally, P12 carried the first duplication of 193 bp comprising exon 6 and part of introns 5&6. Surprisingly, P12 had a moderate AT deficiency (75%) that can be explained by an alternative splicing of the duplicated exon.

Conclusion: Aberrant splicing underlies high numbers of cases with AT deficiency with different consequences: type I, type II or even moderate deficiency. Our data encourage exploring other aberrant splicing as a mechanism involved in AT deficiency, such as mutations affecting exonic splicing enhancers or silencers, and those activating new cryptic exons in intronic regions.

Disclosure of Interest: None declared.

Fibrinogen and Fibrin I

OR371

Interaction of fibrin with the VLDL receptor on endothelial cells: localization of the fibrin-binding site and identification of its inhibitors

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Background: Besides its prominent role in hemostasis, fibrin(ogen) participates in various processes including inflammation. We found that fibrin interacts with endothelial VLDL receptor (VLDLR) through its β N-domains and this interaction promotes transendothelial migration of leukocytes. This discovery suggests a novel fibrin-VLDLR-dependent pathway of leukocyte transmigration and thereby inflammation in which fibrin-VLDLR interaction plays a key role and which may contribute to the pathogenesis of inflammation-related cardiovascular diseases.

Aims: The major aims of this study were to localize fibrin-binding sites in VLDLR and develop efficient inhibitors of this interaction that may be potent anti-inflammatory agents.

Methods: VLDLR fragments were expressed in *Drosophila* and *E. coli* expression systems. Their interaction with fibrin and the $(\beta 15-66)_2$ dimer corresponding to a pair of fibrin β N-domains was tested by ELISA, SPR, and fluorescence spectroscopy.

Results: We prepared soluble extracellular portion of VLDLR (sVLDLR) and its sub-fragments, VLDLR (1–8) and des (1–8) VLDLR, containing eight CR-domains and the rest of the extracellular portion, respectively. Binding experiments revealed that only sVLDLR and VLDLR (1–8) interact with fibrin β N-domains, indicating that the fibrin-binding site is located within the CR-domains. To further localize this site, we prepared recombinant fragments containing CR-domains 1–4 and 5–8, as well as 1–2, 2–3, 3–4, and 2–4, and tested their interaction with the β N-domains. Among these fragments, only those containing CR-domains 1–4, 2–3 and 2–4 exhibited interaction with the β N-domains with K_d of 3.7, 9.5, and 4.6 nM, respec-

tively. These fragments also efficiently inhibited binding of sVLDLR to fibrin.

Conclusion: The results obtained indicate that the fibrin-binding site is located within the 2nd and 3rd CR-domains of VLDLR, the presence of 4th CR-domain increases its affinity, and recombinant fragments containing these domains are potent inhibitors of fibrin-VLDLR interaction.

Disclosure of Interest: None declared.

OR372

Characterizing reactive glutamines in fibrinogen alpha C (233–425) and probing factor XIII substrate specificity

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Background: The C-terminal portion of the Fibrinogen α C chain contains the α C region (221–610). Within Fibrinogen α C (233–425), there are three reactive glutamines (Q) that can be crosslinked by Factor XIIIa to reactive lysines (K) located in the more C-terminal α C domain on another fibrin molecule. Crosslinking of the α C region modifies fibrin clot structure by enhancing lateral aggregation and increasing resistance to fibrinolysis. A greater understanding of the reactivity and substrate specificity of Fbg α C (233–425) will aid in developing new strategies to control fibrin clot architecture and help explain the consequences of genetic truncations or substitutions.

Aims: This study was aimed at characterizing the ability of FXIIIa to cross-link reactive glutamines (Q237, Q328 and Q366) in Fbg α C (233–425) to a set of lysine mimics. The kinetic consequences of replacing selective glutamines with non-reactive residues were explored. Results obtained with this Fbg α C segment were then compared with other FXIIIa substrates.

Methods: A MALDI-TOF mass spectrometry assay and 2D ¹⁵N-¹H HSQC NMR spectroscopy methods were applied to rank each reactive glutamine in α C (233–425). Lysine mimics included glycine ethyl ester and NH₄Cl. SDS-PAGE coupled with fluorescent probes were used to examine FXIIIa's substrate specificity.

Results: The most reactive glutamine Q237 was completely crosslinked to the lysine mimic in < 5 min, followed by Q366 and Q328. The transglutaminase reaction was not dependent upon Q237 and the remaining reactive glutamines Q328 and Q366 could still exhibit independent effects. Fluorescently labeled dansylcadaverine and α_2 -antiplasmin (1–15) show unique and comparable crosslinking events for intact human fibrinogen vs. Fbg α C (233–425).

Conclusion: Reactive glutamines in Fbg α C (233–425) can be ranked as Q237 > Q366 ≥ Q328. Factor XIIIa is not reliant on crosslinking Q237 before proceeding to Q328 and Q366. Fbg α C serves as a valuable model system for exploring FXIIIa substrate specificity.

Disclosure of Interest: None declared.

OR373

Development and validation of a Java-based computer program for image analysis of fibrin ultrastructure

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Background: Quantitation of fibrin clot (CLOT) images obtained from scanning electron microscopy (SEM) is tedious and operator-dependent. We previously reported that the ultrastructure of CLOT could be quantitated by 3 parameters: porous size, fibril diameter and number (#) of fibrils. Hereby, we further automated this process by computing.

Aims: To develop a computer program analyzing SEM images of CLOT.

Methods: Gold sputter-coated CLOT was prepared according to the standard protocol for SEM examination. Images were taken at 20,000×

Each image was enhanced for full 16-bit tonal range with Adobe Photoshop (PS). The porous size of CLOT was computed by PS as the proportion of black pixels at a predefined threshold. The remaining 'non-black' pixels of the image were adjusted with 'filters' in PS to augment the fibril edges. We developed a Java-based program to scan the image, pixel wise, to find the transition from black to white, which represented the fibril edge. Along those edges, the fibril diameter was computed by the # of pixels across the fibril in the direction with the shortest distance. Then, the # of overlapping fibrils at each scanning line was analyzed by the image structure of overlapping fibrils, from which the fibril # was estimated by the adding the fibril edge # to the double of interacting fibrils #.

The accuracy of computing data was validated by three sets of images: (1) pilot images to compare with manual analysis, (2) positive control images from experiments known to form different CLOT ultrastructure and (3) negative control images.

Results: There was no significant difference between computing and manual data in pilot images. For positive controls, the software identified significant differences in all 3 parameters, but no difference was detected in the negative controls.

Conclusion: The newly developed, stand-alone software, combined with PS can objectively quantitate the SEM images of CLOT. Results are comparable to those obtained from manual analysis, but the process takes less than a second.

Disclosure of Interest: None declared.

OR374

Effect of fibrinogen gamma' peptide on factor XI activation by thrombin

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Background: Elevated coagulation factor XI (FXI) is a risk factor for venous thrombosis, while FXI deficiency is a mild bleeding disorder. This makes FXI(a) a promising target for anticoagulant therapy.

Thrombin contributes to FXI activation, particularly at low procoagulant stimuli. FXI activation by thrombin is enhanced by negatively charged surfaces, such as platelet polyphosphate (PolyP), which binds to thrombin exosite II. Since the C-terminus of fibrinogen γ' chain also binds to exosite II and inhibits thrombin's procoagulant activities, we hypothesized that the fibrinogen γ' peptide could interfere with FXI activation.

Aims: To study the effect of the fibrinogen γ' peptide on FXI activation by thrombin.

Methods: FXI activation by thrombin in the presence of 0–250 μ M doubly sulfated peptides with the wild-type (WT-S12) or scrambled (SCR-S12) γ' sequence was studied in model systems by determining chromogenic FXIa activity in timed subsamples. Thrombin-triggered thrombin generation was measured in contact activation-free platelet-rich and platelet-poor plasma in the absence and presence of anti-FXI antibodies.

Results: In the model system, FXI was activated by thrombin at a very slow rate, which was greatly increased by dextran sulfate or PolyP. The fibrinogen γ' peptide inhibited thrombin-mediated FXI activation in a specific and dose-dependent manner, both in the absence of surface (IC_{50} 0.43 μ M for WT-S12 vs. 458.52 μ M for SCR-S12) and in the presence of 100 nM PolyP (IC_{50} 4.05 μ M for WT-S12 vs. 95.72 μ M for SCR-S12). Thrombin-triggered thrombin generation was almost fully FXI-dependent and only observed in platelet-rich plasma, suggesting an essential role of platelets in thrombin-mediated FXI activation in plasma. Peptide experiments in plasma are still ongoing.

Conclusion: The fibrinogen γ' peptide inhibits FXI activation by thrombin both in the presence and in the absence of negatively charged surfaces. This is yet another anticoagulant property of this peptide.

Disclosure of Interest: None declared.

OR375

Fibrin(ogen) engagement of integrin MAC-1 (alpha-M beta-2) limits chronic liver fibrosis induced by a bile duct toxicant in mice

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Background: Chronic liver disease and fibrosis in humans and animal models is associated with activation of the blood coagulation cascade and deposition of cross-linked fibrin in the liver. Previous studies from our laboratory suggest that fibrin inhibits liver fibrosis caused by chronic experimental biliary injury. However, the mechanisms mediating this protective effect are not fully understood.

Aims: The aim of this study is to understand the mechanism underlying the protective effects of fibrin in liver fibrosis. Here, we tested the hypothesis that fibrin inhibits biliary fibrosis through a mechanism requiring its binding to the leukocyte integrin $\alpha_M\beta_2$.

Methods: Biliary injury and fibrosis were induced in Fib $\gamma^{390-396A}$ mice which express a mutant form of fibrinogen that has full clotting function but is incapable of binding integrin $\alpha_M\beta_2$, by feeding chow containing the bile duct toxicant α -naphthylisothiocyanate (ANIT) (0.025%) for 4 weeks.

Results: Liver fibrosis was significantly increased in ANIT-treated Fib $\gamma^{390-396A}$ mice compared to control ANIT-treated wild-type mice, as indicated by increased COL1A1 mRNA levels and increased peribiliary collagen deposition. Interestingly, this increase in liver fibrosis occurred without an associated increase in hepatic parenchymal cell injury. In separate studies, treatment of ANIT-treated wild-type mice with leukadherin-1 (LA-1) (0.4 mg kg⁻¹ day⁻¹, i.p, bid), a small molecule integrin agonist that allosterically enhances $\alpha_M\beta_2$ -dependent cell

adhesion to fibrin, significantly reduced COL1A1 mRNA levels and reduced peribiliary collagen deposition. In contrast, LA-1 treatment had no effect on established liver fibrosis in Fib $\gamma^{390-396A}$ mice.

Conclusion: Collectively, the results indicate that fibrin engagement of integrin $\alpha_M\beta_2$ inhibits experimental xenobiotic-induced biliary fibrosis.

Disclosure of Interest: None declared.

Fibrinolytic system – I

OR376

Nanoparticle-based, molecular control of the plasmin active site to achieve direct fibrinolysis in a novel *in-vitro* system of pulmonary embolism

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Background: Direct fibrinolysis by plasmin has potential for controlled intravascular clot digestion. Protection from neutralization by α_2 anti-plasmin (AP) prior to arrival to clot surface remains a barrier to effective clinical use of plasmin. α_2 AP binds to plasmin's serine protease active site.

Aims: We hypothesize that a plasmin congener can lyse clots when delivered using a nanoparticle displaying a ligand specific to the plasmin active site to block α_2 AP binding.

Methods: A deletion mutant of plasmin, δ -plasmin, lacking kringles 2–5, but retaining the fibrin-binding kringle 1, was synthesized in an *e coli* expression vector and purified by affinity chromatography. Dixon plots of 5 analogues of the serine protease inhibitor benzamide, revealed suitable K_i values of 4 carboxybenzamide (4CB, 301 ± 192 μ M) for δ -plasmin (PMID 25576865). 4CB was conjugated with lipids (DSPE-PEG 2000, Avanti) and sonicated to yield micelles of 9 ± 2 nM diameter. δ -plasmin (10 mg, activity standardized against S2251 chromogenic substrate) was added in 1:2 molar ratio relative to 4CB. Fibrinolysis was then tested in novel *in-vitro* lung model comprising a reservoir, pump, heater, inflow manometer and a 3-D printed, transparent polycarbonate 32-channel parallel circuit, recirculated with human plasma. This 'lung' was pre-embolized with ≈ 4 g of type-matched whole blood thrombi (4×20 mm), and net % lysis was assessed at 60 min by change in clot mass. Positive controls included perfusion with excess tPA to activate all plasminogen in plasma and δ -plasmin in PBS crystalloid buffer devoid of all neutralizing proteins.

Results: Time controls with no δ -plasmin yielded $25 \pm 4\%$ lysis, naked δ -plasmin in plasma produced $52 \pm 5\%$ lysis, whereas tPA in plasma produced $72 \pm 7\%$ lysis, similar to the $72 \pm 2\%$ lysis produced by δ -plasmin in PBS. The 1:2 δ -plasmin:DSPE-PEG 2000-4CB micelles yielded $48 \pm 4\%$ lysis.

Conclusion: Direct fibrinolysis with δ -plasmin can be achieved using a nanoparticle-bound small molecule inhibitor.

Disclosure of Interest: None declared.

OR377

Effect of Quebec platelet disorder on leukocyte urokinase plasminogen activator (uPA) levels

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Background: Quebec Platelet Disorder (QPD) is an inherited bleeding disorder with a unique gain-of-function defect in fibrinolysis, associated with marked overexpression of PLA₂ by megakaryocytes, with-

out systemic fibrinolysis. The molecular cause is a tandem duplication mutation of a large region of chromosome 10 containing PLAUI.

Aims: To determine if QPD markedly increases uPA levels in the leukocytes in blood, namely granulocytes, monocytes and T-lymphocytes.

Methods: Leukocytes were affinity purified from QPD ($n = 6$) and control (C; $n = 7$) subjects using immunomagnetic techniques. Enzyme-linked immunosorbent assay (ELISA) were used to assess uPA and von Willebrand factor (VWF) in each donor's leukocyte and platelet lysates, using VWF levels in these cells to determine the degree of platelet uPA contamination. After excluding samples with $\geq 20\%$ platelet uPA contamination, fold increases (based on means), medians and ranges were determined. To correct for increases from one extra gene copy, results of QPD samples were compared to both 1.0 and 1.5 times the data for control samples, using Mann-Whitney t -tests.

Results: In all subjects, granulocytes contained more uPA than monocytes. Although there were marked increases in uPA in QPD platelets (fold increase > 500 ; pg 10^{-6} cells: Q: median 150, range 38–390; C: median 0.083, range 0.13–0.41; P values: $1 \times < 0.004$, $1.5 \times < 0.004$), QPD granulocytes and monocytes showed only modest increases in uPA (granulocytes 2.4 fold increase; pg 10^{-6} cells: QPD: median 1200, range 770–2000; controls: median 700, range 240–1400; P values: $1 \times < 0.02$, $1.5 \times = 0.45$) (monocytes 4.6 fold increase; pg 10^{-6} cells: Q: median; 560, range; 260–1100; C: median 80, range 50–110; P values: $1 \times < 0.002$, $1.5 \times < 0.01$). Much lower levels of uPA were detected in T-lymphocytes from all donors (ranges: undetectable – 65 pg 10^{-6} cells).

Conclusion: QPD has much more striking effects on platelet than leukocyte uPA levels, further implicating megakaryocyte/platelet-specific mechanisms in the pathogenesis of QPD.

Disclosure of Interest: None declared.

OR378

Assessment of fibrinolytic parameters in patients with hemophilia A

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Background: The idea that fibrinolysis plays a role in the bleeding phenotype of hemophilia A (HA) patients is not new, but the mechanism of enhanced fibrinolysis has not been fully elucidated.

Aims: The aim was to examine the possible increased fibrinolysis in HA patients compared to healthy controls by measuring plasma activities/concentrations of most important fibrinolytic parameters.

Methods: We measured activities of FXIII (Berichrom FXIII), plasminogen (Plg, Berichrom Plasminogen), plasmin inhibitor (Berichrom Antiplasmin), plasminogen activator inhibitor-1 (Berichrom PAI-1, Siemens Healthcare Diagnostics) and thrombin-activable fibrinolysis inhibitor (TAFI, Stachrom TAFI, Diagnostica Stago) in plasma samples of 81 HA patients (37 severe and 44 non-severe) and 101 healthy male controls. In addition, activated and inactivated TAFI antigen concentrations (TAFIa/ai, Asserachrom, Diagnostica Stago) were determined.

Results: Statistically significantly lower Plg (median 99.7%, range 60.3–206.9%, $P = 0.004$) and TAFIa/ai (median 22.9 $\mu\text{g L}^{-1}$, range 10.5–56.5 $\mu\text{g L}^{-1}$, $P = 0.010$) were observed in HA patients compared to healthy controls (Plg mean $\pm$ SD 108.9 $\pm$ 18.5%; median TAFIa/ai 24.5 $\mu\text{g L}^{-1}$, range 14.7–55.1 $\mu\text{g L}^{-1}$). Significantly lower FXIII was identified in severe (mean $\pm$ SD 90.0 $\pm$ 21.5%, $P = 0.014$) than in non-severe HA patients (mean $\pm$ SD 103.8 $\pm$ 27.2%). Regarding TAFI, lower activities were observed in HA patients than in healthy controls, as well as in severe compared to non-severe HA patients, although this difference was not statistically significant ($P = 0.082$ and 0.072, respectively).

Conclusion: Lower TAFIa/ai suggests impaired TAFI activation due to seriously impaired thrombin generation, suggesting enhanced fibrinolysis in HA patients that may contribute to the bleeding tendency. Additionally, observed lower FXIII activities in severe HA patients can possibly contribute to accelerated fibrin clots lysis, rendering hyperfibrinolysis more pronounced in severe as compared to non-severe HA patients.

Disclosure of Interest: None declared.

OR379

Defibrotide improves fibrinolysis efficiency in tPA-resistant arterial thrombosis

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Background: Recombinant tissue-type plasminogen activator (tPA) remains the only approved treatment for acute ischemic stroke. Defibrotide, a polydisperse mixture of single stranded DNA, is an antithrombotic and profibrinolytic drug successfully used in hepatic veno-occlusive disease.

Aims: To evaluate if defibrotide could enhance tPA fibrinolytic efficiency *in vitro* and *in vivo*, using experimental stroke models in mice.

Methods: We studied *in vitro* the effect of defibrotide on plasmin and tPA activities, and performed clot-lysis and binding assays. Later, we assessed defibrotide efficiency in two ischemic stroke models involving either tPA-sensitive or tPA-resistant thrombi. We also explored the effect of defibrotide in diabetic mice. Then, we studied defibrotide potential pro-haemorrhagic side effects *in vivo*.

Results: *In vitro*, defibrotide dramatically accelerated fibrinolysis in the presence of tPA, by enhancing plasmin activity. Interestingly, defibrotide protected neurons from the pro-excitotoxic effect of tPA. Binding studies revealed a strong affinity of defibrotide for tPA (KD = 23.8 $\pm$ 3.2 nM) and plasminogen (KD = 4050 $\pm$ 139 nM). *In vivo*, defibrotide did not further enhance tPA efficiency when the thrombi were already sensitive to tPA alone (*in situ* thrombin-induced stroke model). However, defibrotide made tPA highly effective in stroke models involving tPA-resistant thrombi. Indeed, defibrotide improved outcome in a FeCl₃-induced stroke model and in the thrombin model performed in diabetic mice (in terms of recanalization and lesion size reduction). Importantly, defibrotide did not show significant pro-haemorrhagic effect in a intracranial haemorrhage model (collagenase).

Conclusion: Defibrotide accelerates tPA-mediated fibrinolysis *via* an enhancement of plasmin activity and makes tPA effective in mouse stroke models resistant to fibrinolysis. Defibrotide is a promising adjunct treatment for stroke patients presenting tPA-resistant thrombi, such as diabetic patients.

Disclosure of Interest: None declared.

OR380

Valproic acid selectively increases endothelial, but not brain, t-PA production and reduces thrombus formation in mouse

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Background: The endogenous fibrinolytic system has rarely been considered as a target to prevent thrombotic disease. Tissue-type plasminogen activator (t-PA) is potently increased by histone deacetylase (HDAC) inhibitors in endothelial cells *in vitro*, but whether this translates into increased vascular t-PA production and an enhanced fibrinolytic capacity *in vivo* is unknown. t-PA is also expressed and regulated in most cell types in the central nervous system (CNS), where it performs many roles unrelated to classical fibrinolysis.

Aims: In this study we explored the effect of the clinically used HDAC inhibitor valproic acid (VPA) on t-PA production in the mouse vasculature and brain, and investigated whether VPA affected fibrin deposition and clot formation after mechanical vessel injury.

Methods: Mice were treated with VPA for 96 h after which t-PA mRNA in aorta and brain cortex as well as plasma t-PA levels were measured using real-time PCR and ELISA, respectively. High resolution intravital confocal microscopy was used to quantitate fibrin accumulation and thrombus size following mechanical vessel injury.

Results: We found that low doses of VPA stimulate production of vascular t-PA *in vivo* in mouse and the newly produced t-PA is retained in storage pools in endothelial cells. Further, our findings indicate that, following VPA treatment, this increase of releasable t-PA translates into an antithrombotic effect characterized by reduced fibrin accumulation and smaller thrombi in response to vascular injury. Furthermore, our data suggest that considerably higher concentrations of VPA are required to stimulate t-PA production in the brain compared to the vascular compartment.

Conclusion: Taken together these data suggest that VPA is profibrinolytic and antithrombotic in the vasculature and confers a minimal risk for adverse effects arising from t-PA stimulation in the CNS.

Disclosure of Interest: None declared.

Venous thromboembolism – therapy

OR381

Patients with cancer associated thrombosis: long-term VTE recurrences and bleedings according to cancer site; data from the RIETE Registry

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Background: LMWH are recommended by international guidelines for a period of 3–6 months in patients with cancer associated venous thromboembolism (VTE). The optimal therapeutic option beyond 6 months is not clearly defined. Long-term anticoagulation is most often suggested because of the persisting risk of recurrent VTE but data are very sparse and comprise all types of cancer.

Aims: To assess according to the type of cancer the rate of recurrent VTE, major bleeding and death at 12 months in patients who experienced VTE in a context of active cancer (breast, prostate, colo-rectal, lung) and receiving anticoagulation for at least 3 months.

Methods: We used data from the Registro Informatizado de Enfermedad TromboEmbólica (RIETE) registry, an ongoing international, multicentred, real-life prospective registry of consecutive patients presenting with symptomatic, acute VTE. All patients provided written or oral consent for participation in the registry. Outcome measures included symptomatic VTE, major bleeding, death, type of cancer, and antithrombotic medication.

Results: Up to November 2013, 938, 629, 1189, 1191 patients with respectively breast, prostate, colo-rectal, lung cancer met the inclusion criteria. The highest risk of recurrent VTE occurred in the first 3 months (respectively 1.9%, 1.7%, 2.9%, 6.6%) and decreased thereafter. However the risk of major bleedings at 3 months was almost high (respectively 2.3%, 4.8%, 5.0%, 3.2%) and kept stable thereafter. In terms of mortality, the risk was again highest in the 3 months following the index VTE (respectively 11%, 12%, 19%, 39%) and remained quite stable over time thereafter.

Conclusion: We provide real life data about long-term events in four populations of patients with cancer. We confirm that the long-term recurrence is quite different according to the site of cancer. In addition, our results outline the highest risk of VTE recurrence in the 3 months following the VTE index and the similar risk for VTE recurrence and major bleeding thereafter whatever the cancer.

Disclosure of Interest: None declared.

OR382

Duration of anticoagulation following acute venous thromboembolism in routine care in England: an observational study using CPRD-HES databases

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Background: Current UK (NICE) and International (ACCP) guidelines recommend a minimum of 3 months anticoagulation (AC) following acute venous thromboembolism (VTE). Several models have been proposed to identify patients at high risk of VTE recurrence who should be considered for long-term AC, but these are not widely used in the UK and require external validation.

Aims: This observational study describes AC treatment patterns following acute VTE in England.

Methods: We undertook a retrospective cohort study of all VTE events identified in the UK Clinical Practice Research Datalink linked to Hospital Episode Statistics database (CPRD-HES) from April 1st 2008 to March 31st 2012. VTE occurrence [including pulmonary embolism (PE) and deep vein thrombosis (DVT)] was defined by a VTE code (read or ICD-10 code) with AC prescription within 45 days following the event or hospital discharge. We calculated the cumulative incidence of discontinuation following VTE (accounting for death as a competing event).

Results: Of 11,353 VTE events identified, 62.5% were unprovoked and 37.5% were provoked, including 16.8% in patients with active cancer. Patients' mean age was 63.9 years and 48.5% of VTE events occurred in men. 45.0% of VTE events were PE. Median AC duration was 213 days (95%CI: 212–215). Median AC durations for PE and DVT were 231 (227–234) and 195 (191–198) days, respectively. The cumulative incidence of discontinuation at 3, 6, 12, and 24 months was 7.2%, 26.9%, 73.6%, and 83.5% for PE; and 13.8%, 45.9%, 82.8% and 90.1% for DVT.

Conclusion: Although a minimum of 3 months AC following acute VTE is recommended, our results suggest that clinicians prefer to manage VTE for a minimum of 6 months (~ ½ of DVT and ~ ¾ of PE patients remained anticoagulated at this point). The majority of VTE patients discontinued AC within 12 months of diagnosis. The observed variation in AC treatment behavior shows the current variability in adherence to clinical VTE guidelines in England.

Disclosure of Interest: L. Lacoin Employee of: Bristol-Myers Squibb, E. Ridha Employee of: Bristol-Myers Squibb, C. Lefevre Employee of: Bristol-Myers Squibb, V. Moorthy Consultant for: Bristol-Myers Squibb, M. Vasudev Consultant for: Bristol-Myers Squibb, S. Lister Employee of: Bristol-Myers Squibb, A. Bird Employee of: Pfizer, I. Minns Employee of: Bristol-Myers Squibb, A. Bakhai Consultant for: Bayer, Bristol-Myers Squibb, Pfizer, Boehringer Ingelheim, Daiichi Sankyo, R. Alikhan Consultant for: Bayer, Bristol-Myers Squibb, Pfizer, Boehringer Ingelheim, Daiichi Sankyo.

OR383

No beneficial effect of statins in reducing recurrent disease in a meta-analysis of 21,587 patients with acute symptomatic venous thromboembolism treated in phase 3 trials comparing direct oral anticoagulants with vitamin K antagonists

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Background: Recent studies have suggested a reduced risk of recurrent venous thromboembolism (VTE) with statins.

Aims: To confirm the beneficial effect of statins on recurrent disease in a large homogeneous cohort, by conducting a meta-analysis of statins in phase 3 trials comparing a direct oral anticoagulant (DOAC) with standard vitamin K antagonist (VKA) therapy in patients with acute symptomatic VTE.

Methods: Statin use at baseline was analyzed in all patients in the RECOVER I+II (dabigatran etexilate), EINSTEIN-DVT and -PE (rivaroxaban), and HOKUSAI trials (edoxaban). Outcomes were similarly defined across studies and centrally adjudicated. Effect of statins was analyzed in all patients pooled using cumulative incidences and the Mantel-Haenszel method with random effects, and per individual study.

Results: Statin use at baseline was retrieved for 21,587 of 21,726 included patients; 2754 (12.8%) used statins and 18,833 (87.2%) did not. Statins had no effect on the risk of recurrent VTE or VTE-related death in the pooled analysis (odds ratio [OR] 1.03; 95% confidence interval [CI] 0.74–1.44). Results were similar for recurrent DVT (OR 1.15; 0.81–1.64) and recurrent PE (OR 1.04; 0.74–1.46). Analyses of the individual studies were consistent with the pooled analysis. Hazard ratios in the individual studies, adjusted for age, sex, creatinine clearance < 50 mL min⁻¹, diabetes mellitus, hypertension, prior VTE, and aspirin use, remained similar. A sub-analysis in patients with DOAC or VKA therapy separately showed more benefit of statins with DOACs (OR 0.78; 0.49–1.22) than with VKAs (OR 1.36; 0.96–1.93) on the risk of recurrent VTE or VTE-related death. This was similar for recurrent DVT (OR 0.67; 0.33–1.38 vs. OR 1.68; 1.11–2.54) and recurrent PE (0.91; 0.55–1.52 vs. OR 1.22; 0.78–1.91).

Conclusion: We could not confirm the beneficial effect of statins on recurrent disease in 21,587 patients treated in phase 3 acute VTE trials. Further analyses on discrepancies between DOAC and VKA-treated patients are ongoing.

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OR384

Net clinical benefit of hospitalization vs. outpatient management of patients with acute pulmonary embolism

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Background: The decision to hospitalize or not patients with acute pulmonary embolism (PE) is controversial. To consider outpatient treatment, numerous studies have focused on identifying patients who are at low risk of adverse events. However, the hospitalization on itself may lead to in-hospital complications and potentially worsen the prognosis of PE patients.

Aims: To determine the net clinical benefit of hospitalization vs. outpatient management of patients with acute PE.

Methods: Retrospective cohort study of patients presenting with PE, from 1 January 2007 to 31 December 2012, at the Ottawa Hospital where usual practice is to consider outpatient treatment for most of the patients who do not need specific in-hospital care. Patients treated as outpatient or inpatient were propensity score matched to balance out differences for 25 patient characteristics and known risk factors of adverse events including PESI criteria, troponin level and right ventricular dilatation. The primary outcome was the rate of adverse events at 14 days including recurrent venous thromboembolism (VTE), major bleeding or death.

Results: Among 1143 eligible patients, 556 were included in the matched cohort, 278 in each group. The 14-day rate of adverse events was 1.1% for outpatients and 12.5% for inpatients: adjusted OR: 17.9 (95%CI: 4.9–65.2). The rates of recurrent VTE, major bleedings and deaths were: 0.4% vs. 2.2% ($P = 0.03$), 0% vs. 4.3% (-) and 0.7% vs. 7.5% ($P < 0.001$), respectively.

The 3-month rate of adverse events was 5.4% for outpatients and 21.3% for inpatients: OR 5.5 (95%CI: 2.9–10.4). In the subgroup of patients with simplified PESI > 0 ($n = 115$), the 14-day rate of adverse events was 3.4% vs. 14.3%: OR 4.7 (0.96–23.4) and the 3-month rate was 10.2% vs. 29.1%: OR 3.6 (1.3–10.1) among outpatients and inpatients.

Conclusion: Outpatient treatment of PE patients seems associated with a lower rate of adverse events than hospitalization and may be considered as first-line management.

Disclosure of Interest: None declared.

OR385

Extended durations of anticoagulation and mortality following incident venous thromboembolism in the Q-VTE cohort

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Background: Extended duration of anticoagulation following incident venous thromboembolism (VTE) may impact patient survival, however, data are limited.

Aims: To determine the effect of anticoagulant treatment duration on all-cause mortality in patients with first acute VTE.

Methods: Using the linked healthcare administrative databases of the province of Québec, Canada, we identified all patients with incident VTE between 2000 and 2009. Patients who died or were not dispensed an anticoagulant in the 14-day period following the incident VTE were excluded. Treatment duration was determined for continuous anticoagulant use. All-cause mortality was defined as any death occurring 14 days or more following VTE. Time-dependent Cox proportional hazards models were used to estimate adjusted hazard ratios (HR) with 95% confidence intervals (CI) of all-cause mortality associated with extended durations of anticoagulant use compared with short-term use (0–3 months).

Results: A total of 30,366 patients met inclusion criteria and 27.2% had active cancer. During a mean follow-up of 3.4 years, 8278 deaths occurred. Vitamin K antagonists (VKA) were dispensed to 90.9% of patients. Median duration of continuous anticoagulant use was 61 days (interquartile range, 36–119 days). Short-term users comprised 64.8% of the patients, whereas durations of use for 3–6, 6–12, and > 12 months were less frequent (20.7%, 10.7% and 3.9%, respectively). Compared with short-term use, extended durations of continuous anticoagulant use were associated with reduced all-cause mortality (HR: 0.79, 95% CI: 0.75–0.84; HR: 0.71, 95% CI: 0.65–0.76; HR: 0.82, 95% CI: 0.73–0.92, for 3–6, 6–12, and > 12 months, respectively).

Conclusion: In this large population-based cohort of incident VTE, anticoagulant therapy for periods longer than three months was associated with a mortality benefit.

Disclosure of Interest: None declared.

Hemophilia – joint disease and clinical

OR386

Intracranial haemorrhage in children with inherited bleeding disorders in the UK 2003–2013

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Background: Intracranial haemorrhage (ICH) is an uncommon but potentially life-threatening complication of inherited bleeding disorders (IBDs).

Aims: To define the clinical characteristics and management of ICH in children with IBDs attending UK haemophilia centres between 2003 and 2013.

Methods: Questionnaire survey of paediatric haematology specialists at UK haemophilia centres.

Results: Fifty-five cases with ICH were reported from 16 centres. 42 (76%) had haemophilia (41 severe), 2 (4%) vWD and 11 (20%) a rare coagulation disorder. No cases had a platelet function disorder. Haemophilia A (HA) was the most commonly reported disorder and the incidence of ICH in HA children registered with UKHCDO was 6.0 per 1000 patient years at risk (95% CI 4.14–8.60). The median age at

presentation was 4 months (0 days–4.5 years). 31% presented as neonates and 91% at < 2 years of age. In 29 (53%) bleeding was subdural, 22 (40%) intracerebral and 4 (7%) subarachnoid. In neonatal ICH the mode of delivery was spontaneous vaginal in 9; forceps/ventouse in 4; caesarean section in 3 and unknown in 1. Trauma prior to ICH in children age 1 month–2 years occurred in 7/33 (21%) compared to 3/5 (66%) in those aged > 2 years. 4/24 (17%) cases with HA had an inhibitor. 51/55 (95%) of cases were not receiving any form of prophylactic therapy at the time of the event. 18/55 (33%) had surgical treatment of ICH in addition to replacement therapy. The median duration of replacement therapy was 19 days (range 5–42 days). There were 5 reported deaths. Of survivors 20/50 (44%) had neurological sequelae. Overall 17/34 (50%) of surviving children with HA developed an inhibitor following treatment for ICH, including 6/10 (60%) of those treated as neonates.

Conclusion: ICH in children with IBDs occurs most frequently during the first 2 years of life in children with severe disorders not receiving prophylaxis and often occurs in the absence of documented trauma.

Disclosure of Interest: None declared.

OR387

Perioperative treatment of hemophilia A patients: blood group O patients are at risk of bleeding complications

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Background: Perioperative administration of clotting factor VIII concentrate in hemophilia A may result in both underdosing and overdosing, associated with the risk of bleeding complications or unnecessary costs, respectively.

Aims: To identify the extent and the predictors of underdosing and overdosing in perioperative management of (moderate) severe hemophilia A.

Methods: Hundred-nineteen hemophilia A patients with FVIII levels < 0.05 IU mL⁻¹ undergoing 198 elective, low or medium risk surgical procedures from five Hemophilia Treatment Centers in the Netherlands between 2000 and 2012 were included (median age 40 years, median body weight 75 kg). Perioperative management was evaluated by quantification of perioperative infusion of FVIII concentrate and achieved FVIII levels. Predictors of underdosing in the first 24 h after surgery and (excessive) overdosing were analyzed by logistic regression analysis. Excessive overdosing was defined as target level plus a deviation of above 0.20 IU mL⁻¹. This study was not subject to the Medical Research Involving Human Subjects Act and approved by the Medical Ethics Committee.

Results: Depending on postoperative day, 7–45% of achieved FVIII levels were under and 33–75% were above predefined target ranges as stated by the National Hemophilia Consensus. An overall reduction of FVIII consumption of 44% would have been attained if target levels had been maintained. Blood group O was predictive of underdosing

(OR = 3.3 95%CI [2.0–5.5]) and these patients had more bleeding complications in comparison to patients with blood group non-O (OR = 2.02 95%CI [1.00–4.09]). Patients with blood group non-O were at higher risk of overdosing (OR = 1.6 95%CI [1.3–2.1]). In addition, patients treated with bolus infusions were at higher risk of excessive overdosing (OR = 1.7 95%CI [1.3–2.2]).

Conclusion: Quality of care and cost-effectiveness can be improved by refining of dosing strategies based on the predictors blood group, age and mode of infusion.

Disclosure of Interest: None declared.

OR388

Spinart 3-year analyses: patient- and joint-level changes in colorado adult joint assessment scale and magnetic resonance imaging scores with Bayer's sucrose-formulated recombinant factor VIII in adolescents and adults

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Background: Efficacy and safety of routine prophylaxis vs. on-demand treatment with Bayer's sucrose-formulated recombinant factor VIII (rFVIII-FS) in patients with severe hemophilia A were evaluated in the randomized, controlled SPINART study.

Aims: Patient- and joint-level changes at year 3 in magnetic resonance imaging (MRI) and Colorado Adult Joint Assessment Scale (CAJAS) scores were compared to investigate if individual joint data revealed results that may have been obscured in previously reported patient-level analyses.

Methods: SPINART included males aged 12–50 years with severe hemophilia A, ≥ 150 exposure days to FVIII, no inhibitors, and no prophylaxis for > 12 months in the past 5 years. Patients were randomized 1:1 to rFVIII-FS on demand or prophylaxis (25 IU kg⁻¹ 3× per week). Changes from baseline to year 3 were evaluated for 6 index joints (knees, ankles, elbows) using the Extended MRI (eMRI) scale and CAJAS. Percentages of patients or joints with improved, unchanged, or worsened scores were evaluated.

Results: Of the 84 patients in SPINART, eMRI and CAJAS change from baseline data were available for 62 (prophylaxis, *n* = 32; on demand, *n* = 30) and 76 patients (*n* = 39; *n* = 37) and for 386 (*n* = 197; *n* = 189) and 446 joints (*n* = 224; *n* = 222). Categorical analysis of CAJAS data at year 3 showed a higher percentage of patients treated prophylactically vs. on demand with improved scores (64.1% vs. 43.2%) and a lower percentage with worsened scores (28.2% vs. 51.4%); with eMRI, the percentage improved was smaller (12.5% vs. 6.7% improved; 75.0% vs. 73.3% worsened). At individual joints, improved, unchanged, and worsened CAJAS scores in patients treated with prophylaxis vs. on demand were 46.0% vs. 33.3%, 22.3% vs. 24.3%, and 31.7% vs. 42.3%; eMRI values were 8.1% vs. 3.7%, 61.9% vs. 69.8%, and 29.9% vs. 26.5%.

Conclusion: These data suggest that in patients with severe hemophilia A, joint function as measured by CAJAS is more likely to improve after 3 years of adult routine prophylaxis with rFVIII-FS than joint structure as measured by MRI.

Disclosure of Interest: S. Funk Consultant for: Bayer-sponsored SPINART study, B. Lundin Consultant for: Bayer HealthCare (has been under contract to Bayer HealthCare for work performed for the cross-sectional study 12,948 and is under contract for work performed for SPINART), Employee of: Center for Medical Imaging and Physiology at the University Hospital of Skåne, W. Hong Employee of: Bayer HealthCare.

OR389

Bleeding phenotype and baseline FVIII level in patients with nonsevere hemophilia A: results from the INSIGHT study

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Background: In order to explore which prophylactic through levels should be targeted for optimal joint protection in patients severe hemophilia A,

Aims: we assessed the annual (joint) bleeding rate according to the endogenous FVIII level (FVIII:C) in patients with nonsevere hemophilia A.

Methods: We performed these analyses in data obtained from the INSIGHT study, an international cohort study including pediatric and adult patients with nonsevere hemophilia (FVIII:C 0.02–0.40 IU mL⁻¹). Within this cohort a nested case control study was conducted (73 inhibitor patients and 234 controls). Data on every FVIII exposure were collected until inhibitor development in cases and up to the same number of exposure days in controls. The annual joint bleeding rates calculated over a life-time period were compared according to the endogenous FVIII:C. Both joint bleeds that occurred after (minor) trauma as well as spontaneously were considered.

Results: A total of 307 patients (214 mild, 93 moderate HA) were included. Patients were followed for a median of 21 exposure days (IQR 11–38) until a median age of 31.0 years (IQR 13.3–54.1). Half of the patients (50%) had experienced at least one joint bleed and 83% at least any type of bleed. The median annual bleed rate was 0.013 per year (IQR 0.00–0.13) for joint bleeds and 0.13 per year (IQR 0.029–0.51) for any bleed. The lifetime cumulative incidence of joint bleeds and median (IQR) annual joint bleed rate according to endogenous FVIII:C was 59.1% and 0.052 (0.00–0.22) for FVIII:C 0.02–0.05 IU mL⁻¹; 48.4% and 0.00 (0.00–0.16) for FVIII:C 0.05–0.10 IU mL⁻¹; 43.4% and 0.00 (0.00–0.082) for FVIII:C 0.10–0.20 IU mL⁻¹; 44.8% and 0.00 (0.00–0.065) for FVIII:C 0.20–0.30 IU mL⁻¹; 54.5% and 0.014 (0.00–0.19) for FVIII:C 0.30–0.40 IU mL⁻¹.

Conclusion: There was an impression towards a more severe bleeding phenotype in patients with FVIII:C 0.02–0.05 IU mL⁻¹, as compared to patients with FVIII:C > 0.05 IU mL⁻¹, however, among patients with FVIII:C > 0.05 IU mL⁻¹ bleeding phenotypes did seem to not depend on endogenous FVIII:C.

Disclosure of Interest: None declared.

OR390

Novel imaging technique for assessment of early cartilage changes in haemophilic knees and ankles

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Background: In hemophiliacs suboptimally treated, the resultant cartilage degeneration increases morbidity and decreases quality of life. Quantitative T2 maps hold potential for detecting early structural changes of cartilage; this is useful in prophylaxis follow-up.

Aims: (i) To determine correlations between T2 maps obtained in different regions-of-interest (ROIs) of ankles and knees; (ii) To determine correlations between single- and multi-echo T2 maps at a single time-point and International Prophylaxis Study Group (IPSG) MRI scores; (iii) To assess intra-/inter-reader reliability of T2 map analysis.

Methods: Twenty-five hemophilic boys (ages 7–18) with different severity levels of chronic arthropathy (16 ankles; 9 knees) were scanned at 3T MRI. Sagittal T2 maps were obtained (TE = 13,19,28 ms). For ankles and knees, respectively 5 and 8 ROIs (weight-bearing [WB] and non-weight-bearing [NWB] areas) were examined using single- and multi-echo maps. Semi-quantitative assessment of cartilage was performed with the IPSG MRI scale. The Canadian Hemophilia Society funded the study.

Results: IPSG score range was 0–7 in ankles, and 0–11 in knees. In ankles substantial correlations were noted between single-echo WB T2 maps and osteochondral (OC) (r range, 0.60–0.68, $P < 0.05$) IPSG scores, and moderate/substantial correlations between multi-echo WB T2 maps and OC (r range, 0.57–0.76, $P < 0.05$) scores. In knees substantial correlations were found between single-echo WB T2 maps and total ($r = 0.65$, $P < 0.05$)/OC ($r = 0.63$, $P < 0.05$) scores. No such correlations were noted in NWB areas with any technique in any joint. Whereas intra-reader reliability of map analysis was substantial/excellent (ICC = 0.71–0.97) for ankles, inter-reader reliability was variable (ICC = 0.27–0.89). In knees intra- and inter-reader reliability were also variable.

Conclusion: Correlations between T2 maps and IPSG scores with either single or multi-echo T2 maps show potential for the technique as a surrogate of cartilage damage. Further longitudinal research on the value of T2 maps is warranted.

Disclosure of Interest: None declared.

Risk factors for venous thrombosis – III

OR391

Ageing of the venous valves as a new risk factor for venous thrombosis in the elderly – the BATAVIA study

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Background: Increasing age is the strongest risk factor for venous thrombosis (VT). Increasing age has been related to a thickening of the venous valves and a decreased valvular function. The association between ageing of the venous valves and the risk of VT is not known.

Aims: To assess the effect of increased valve thickness on the risk of VT.

Methods: Analyses were performed in the BATAVIA study, including 76 patients aged 70+ years with a first deep vein thrombosis of the leg

and 97 controls. We performed an ultrasound examination of the venous valves in the popliteal veins. The non-affected leg represents the stage of the ageing process of the venous valves in the affected leg prior to the thrombotic event. The valves were imaged with a 9 MHz linear probe using B-mode ultrasonography. Valve closure time (VCT) was measured as an indicator for valve function using an automatic inflatable cuff. To estimate the risk of VT, valve thickness was dichotomized at the 90th percentile as measured in controls. Informed consent was obtained and the study was approved by the Medical Ethical Committee of the Leiden University Medical Center.

Results: In 70 of the 76 (92%) patients (ipsi- or contralateral leg) and 93 of the 97 (96%) controls, valve parameters could be measured. Mean valve thickness of the controls was similar in the left and right leg, i.e., left: 0.36 mm (95%CI:0.34–0.37) and right: 0.37 mm (95%CI:0.35–0.39). In 47 patients the non-affected leg could be measured: mean valve thickness: 0.39 mm (95%CI:0.37–0.42). Patients had an increased valve thickness compared with controls: mean difference 0.03 mm (95%CI:0.01–0.06). 11/47 Patients and 8/87 controls a valve thickness exceeding the cut-off value (> 0.44 mm), resulting in an age and sex adjusted OR of 4.1 (95%CI:1.4–12). Results were similar for the ipsilateral leg ($n = 37$). There was no association between valve thickness and VCT.

Conclusion: Increased valve thickness was positively associated with the risk of VT in the elderly.

Disclosure of Interest: None declared.

OR392

Impact of inherited risk factors of venous thrombosis on the associations between family history of myocardial infarction and risks of myocardial infarction and venous thromboembolism

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Background: A family history of myocardial infarction (FHMI) is known to increase the risk of myocardial infarction (MI) and venous thromboembolism (VTE) independent of traditional atherosclerotic risk factors. Even though common mechanisms have not been displayed, it is likely to assume that shared genetic variants are involved.

Aims: To investigate the joint effect of genetic thrombotic risk factors and FHMI on risk of VTE and MI in a case-cohort recruited from a general population.

Methods: Cases with a first VTE ($n = 489$) and an age-weighted subcohort ($n = 1343$) sampled from the Tromsø 4 (1994–1995) and 6 (2007–2008) studies were included. DNA-samples obtained at baseline were genotyped for FV-Leiden (rs6025), prothrombin 20210A (rs1799963), and ABO blood type (rs8176719). Cox regression models were used to calculate hazard ratios (HR) across categories of gene variants and FHMI, and all analyses were adjusted for age, sex and body mass index. The study was approved by the Regional Committee of Research Ethics, and all participants provided informed written consent.

Results: There were 276 and 489 incident MIs and VTEs during a median follow-up time of 16 years. Parental heritage of MI was signifi-

cantly associated with MI (HR 1.60, 95% CI 1.11–2.29), and the risk increased with the numbers of affected relatives. In general, FHMI increased the risk of VTE, particular for parental heritage (HR 1.26, 95% CI 1.02–1.56). The risk of MI and VTE by FHMI did not alter when adjusted for factor V Leiden, prothrombin 20210A, and ABO blood type. There was a moderately increased risk of VTE, but not MI, when the genetic thrombotic risk factors were combined with parental FHMI; HR of VTE associated with FV-Leiden increased from 2.56 (95% CI 1.70–3.86) to 3.95 (95% CI 1.92–8.12).

Conclusion: Our findings suggest that the risk of VTE and MI by FHMI is not caused by FV-Leiden, prothrombin 20210A, and non-O. However, parental FHMI and the genetic risk factors had joint effect on VTE risk, but not MI risk.

Disclosure of Interest: None declared.

OR393

Impact of variants in the factor 5 gene on the risk of venous thromboembolism in cancer

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Background: Venous thromboembolism (VTE) is a common complication in cancer. Factor 5 (F5) acts as an important co-factor in the coagulation system and variants in the F5 gene (rs6025 (FV-Leiden) and rs4524) are independently associated with VTE risk. However, information regarding the impact of these gene variants on VTE risk in cancer patients is limited.

Aims: To investigate the joint effect of F5 SNPs (rs6025 and rs4524) and active cancer on VTE risk in a case-cohort recruited from a general population.

Methods: Cases with a first VTE ($n = 609$) and an age-weighted subcohort ($n = 1691$) sampled from the Tromsø 4 (1994–1995) and 6 (2007–2008) studies were included. Baseline DNA-samples were genotyped. Validated VTEs and incident cancer were registered up to December 31st, 2012. Hazard ratios for VTE were calculated across categories of cancer and gene variants and compared to cancer-free subjects without the risk alleles. Cancer was defined as active cancer (i.e. six months before until one year following a cancer diagnosis). The study was approved by the regional ethical committee, and informed written consent was obtained from all participants.

Results: There were 524 cancer diagnoses and 93 cancer-related VTEs during follow-up. The risk of a cancer-related VTE was 9.8-fold (95% CI 7.8–12.3) higher and increased further in individuals with 1 or 2 risk alleles at rs6025 (HR 19.6, 95%CI 11.2–34.1) or rs4524 (HR 21.1; 95%CI 12.5–35.4). Similarly, the risk of DVT was increased in subjects with F5 rs6025 risk alleles (HR 28.8; 95%CI 15.2–54.6) and rs4524 risk alleles (HR 29.9; 95%CI 13.6–66.1). Individuals with cancer and risk alleles at both loci had a higher risk of VTE (HR 36.8; 95%CI 17.8–76.0) and DVT (HR 69.4; 95%CI 26.3–183.0). The F5 rs4524 SNP explained a higher proportion of the VTE risk in cancer patients than rs6025 (66% vs. 47%).

Conclusion: Our findings imply that cancer patients carrying the F5 rs6025 and/or rs4524 risk alleles are at substantially higher risk of VTE than those carrying non-risk alleles.

Disclosure of Interest: None declared.

OR394

Identification of new genetic risk factors for venous thrombosis by targeted sequencing: results of the MILES study

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Background: Although several genetic risk factors for venous thrombosis (VT) are known, almost all in the coagulation pathway, a large unexplained genetic component remains.

Aims: We aimed to identify new genetic risk factors for VT using targeted sequencing.

Methods: In the Milan Leiden Sequencing (MILES) study, we selected 657 patients with a first VT and 599 controls from three population-based case-control studies (MEGA, THE VTE, and the Milan study). Individuals were selected using the following criteria: European ancestry, no surgery or malignancy, no natural anticoagulant deficiency, and no factor V Leiden or prothrombin 20210A carriership. We performed targeted sequencing of the coding regions, 3', and 5' UTRs of 740 genes involved in thrombosis and hemostasis or closely related pathways. We performed single-variant association tests for all low-frequency and common variants (minor allele frequency (MAF) $\geq 1\%$) and gene-based association tests for all rare variants (MAF $< 1\%$). All analyses were adjusted for age, sex, country, and multiple testing was accounted for by calculation of the False Discovery Rate (FDR).

Results: Single-variant association tests for 5218 common variants showed 123 associations with VT risk (FDR < 0.20). Most of these variants (89%) were located in *F5*, *ABO*, *FGG/FGB/FGA*, and the *F11* region. In addition, we identified 14 VT-associated variants located in 10 genes that, except for *STAB 2*, have not been implicated in VT risk before. The strongest candidates were a non-synonymous variant in *F2RL3* (rs2227376: OR 0.4, 95% CI 0.2–0.7), a non-synonymous variant in *STAB 2* (rs17034433: OR 0.5, 95% CI 0.4–0.8) and a 3'UTR variant in *MASPI* (rs72549167: OR 3.4, 95% CI 1.6–7.3). Gene-based analyses showed a burden of rare variants in 9 genes.

Conclusion: Using a targeted sequencing strategy we were able to identify new genetic risk factors for venous thrombosis. Replication and functional studies are needed to confirm our findings and to unravel the biological mechanisms involved.

Disclosure of Interest: None declared.

OR395

Occurrence and predictors of recurrence within 3 years after a first episode of acute venous thromboembolism: Worcester Venous Thromboembolism Study

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Background: Venous thromboembolism (VTE), comprising deep vein thrombosis (DVT) and pulmonary embolism (PE), has multiple risk factors and tends to recur. Contemporary data from population-based observational studies are limited.

Aims: To quantify the magnitude and identify predictors of recurrence within 3 years after first-time VTE.

Methods: Population-based surveillance study with 3-year follow-up among residents of central Massachusetts (MA), USA, diagnosed with an acute first-time PE and/or lower-extremity DVT from 1999 to 2009 in hospital and ambulatory settings in all 12 central MA hospitals. Medical records were reviewed by trained abstractors and validated by clinicians.

Results: The 2989 study patients were followed for 5836 person-years; mean follow-up duration 23.4 (median 30) months. Mean age was 64.3 years, 44% were men, and 94% were white. The proportions of cancer-associated, provoked, and unprovoked VTE episodes were 17%, 43%, and 40%, respectively. During follow-up, 329 patients developed a recurrent VTE; cumulative incidence rate of recurrent VTE within 3 years after the index VTE was 15% overall, and 25%, 13%, and 13% among patients with active cancer, provoked, or unprovoked VTE, respectively. Predictors (hazard ratios [95%CI]) of recurrent VTE identified by multivariable Cox proportional hazard regression were active cancer with (2.6 [1.9–3.6]) and without chemotherapy (1.6 [1.1–2.4]), hypercoagulable state (2.5 [1.3–4.8]), inferior vena cava filter placement (2.0 [1.5–2.7]), varicose vein stripping (1.7 [1.1–2.7]), superficial thrombophlebitis (1.6 [1.1–2.4]), and surgery within 3 months before the index event (0.7 [0.6–0.9]).

Conclusion: The cumulative incidence of VTE recurrence over 3 years of follow-up remains high among residents of central MA diagnosed with a first episode of VTE. Patients with active cancer are at higher risk for developing a recurrent event compared to those with provoked or unprovoked VTE.

Disclosure of Interest: None declared.

Anti-platelets agents – clinical

OR396

Platelet function as a predictor of ischemic and bleeding events in pad patients undergoing pta: results from Traiano study

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Background: Antiplatelet therapy is a key intervention to reduce vascular events in peripheral artery disease (PAD) patients, but literature data demonstrate that standard antiplatelet therapy is less effective in PAD.

Aims: To assess the degree of on-treatment platelet reactivity, and evaluate its association with ischemic and hemorrhagic adverse events.

Methods: In 177 PAD patients (males 118, females 59; mean age 74.1 ± 10.1) undergoing PTA on dual antiplatelet therapy, we investi-

gated platelet aggregation (PA) by $10 \mu\text{M L}^{-1}$ adenosine diphosphate (ADP), and 1 mM arachidonic acid (AA) on a venous blood sample obtained within 24 h from PTA. High platelet reactivity (HPR) by ADP was defined as $\text{PA} \geq 70\%$, and HPR by AA as $\text{PA} \geq 20\%$.

Results: At a median follow-up of 24 months, we documented 23 (8.7%) deaths, 1 (0.4%) acute myocardial infarctions, 17 (6.5%) restenosis, and 24 (9.1%) TIMI-minor bleedings. At multivariate analysis adjusted for age, sex and cardiovascular risk factors HPR by ADP and AA were significantly associated with death [HPR by ADP: HR 5.4, 95%CI 1.3–22.2; HPR by AA: HR 4.0, 95%CI 1.1–15.2]. PA by ADP values were significantly lower in patients with bleedings [26.5 (IQR 22.0–39.3)% vs. 62.0 (IQR 44.5–74.0)%, $P < 0.001$]. At ROC curve analysis, the cut-off value of PA by ADP associated with increased bleeding risk was 41%. After adjustment for age, sex, body mass index, and renal function, a low platelet reactivity (LPR) (PA by ADP $< 41\%$) was a significant predictor of bleeding.

Conclusion: 1) HPR by ADP and AA are predictors of death; 2) LPR by ADP is predictor of bleeding. This suggests that on treatment platelet reactivity might be used as a clinical tool for optimizing antiplatelet therapy in PAD patients.

Disclosure of Interest: None declared.

OR397

Identification of candidate gene modulating platelet reactivity in aspirin-treated patients using a novel network-based approach

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Background: Platelet reactivity (PR) in cardiovascular (CV) patients is variable between individuals and modulates clinical outcome. However, the determinants of platelet reactivity are largely unknown. Integration of data derived from high-throughput omics technologies may yield novel insights into the molecular mechanisms that govern platelet reactivity.

Aims: The aim of this study was to identify candidate genes modulating platelet reactivity in aspirin-treated cardiovascular patients.

Methods: PR was assessed in 110 CV patients treated with aspirin 100 mg day^{-1} by aggregometry using several agonists. CV patients with extreme high or low PR were selected for further analysis. Quantitative proteomic of platelets and platelet sub-cellular fractions as well as transcriptomic analysis were integrated with a network biology approach. This allowed the identification of gene products differentially expressed in patients with extreme PR phenotypes. Since recent data showed that circulating microRNAs (miRNAs) are associated with PR, we then specifically assessed the level of miRNAs that were differentially expressed in these two groups of patients (Nanostring technology). The predicted targets of these miRNAs were mapped onto the network to identify the most relevant genes.

Results: We first constructed a network gathering platelet proteomic and transcriptomic data associated to extreme PR phenotypes. It yielded a network of 99 nodes and 309 edges, representing platelet activation pathways. Five miRNAs were identified as differentially expressed and associated with platelet reactivity. Among these 5 miRNAs, 2 are predicted to have an identical gene target whose gene expression is up-regulated in patients with extreme high PR compared to patients with extreme low PR.

Conclusion: The integration of several 'omics datasets allowed the identification of candidate genes that may be associated with the modulation of platelet reactivity in aspirin-treated cardiovascular patients.

Disclosure of Interest: None declared.

OR398

Prasugrel monotherapy provides most effective platelet inhibition without affecting fibrin clot dynamics in individuals with diabetes

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Background: The cellular and protein phase of coagulation contribute to the formation of a platelet-rich fibrin clot. Diabetes is characterised by an enhanced thrombotic environment secondary to tight fibrin networks, impaired fibrinolysis and increased platelet reactivity. Despite the increased thrombosis risk in diabetes, long-term antiplatelet therapy remains similar to the non-diabetic population.

Aims: Compare monotherapy with aspirin, clopidogrel or prasugrel on fibrin clot structure/fibrinolysis and platelet activation in type 2 diabetes (T2DM).

Methods: A total of 56 T2DM patients, on stable aspirin monotherapy, completed a placebo-controlled, double-blind cross-over study using instead monotherapy with clopidogrel or prasugrel. Thrombosis potential was assessed as fibrin clot final turbidity/lysis time and platelet aggregation response to different agonists.

Results: Clot maximum absorbance (mean $\pm$ SD) on aspirin, clopidogrel and prasugrel was 0.238 ± 0.080 , 0.230 ± 0.086 and $0.236 \pm 0.083 \text{ au}$ ($P > 0.1$) with no difference detected in clot lysis time (520 ± 112 , 522 ± 133 and $522 \pm 101 \text{ s}$).

Platelet aggregation induced by $5 \mu\text{M}$ ADP was lower during prasugrel (27.9 ± 12.8) compared with clopidogrel therapy ($49.2 \pm 16.8 \text{ au}$; $P < 0.001$), and both were lower than aspirin ($71.1 \pm 8.9 \text{ au}$; both $P < 0.001$). Platelet aggregation induced by $2 \mu\text{g mL}^{-1}$ collagen was lower during prasugrel therapy (60.2 ± 18.5) compared with clopidogrel ($70.5 \pm 19.0 \text{ au}$, respectively; $P < 0.01$) and response to arachidonic acid stimulation was also reduced (52.6 ± 31.1 and 61.1 ± 36.1 , respectively; $P < 0.01$). P-selectin expression induced by $10 \mu\text{M}$ ADP was reduced with prasugrel compared with clopidogrel (11.5 ± 8.1 and 24.8 ± 17.2 , respectively, $P < 0.001$).

Conclusion: Monotherapy with prasugrel in diabetes is associated with stronger inhibition of platelet function compared with clopidogrel and a broader range of inhibition of platelet responses compared with aspirin. Outcome studies are warranted to investigate the role of long-term monotherapy with prasugrel in diabetes.

Disclosure of Interest: None declared.

OR399

Generation and characterization of a panel of nanobodies towards human MMP-2: application to the study of MMP-2 in cardiovascular pathophysiology

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Background: MMP-2, a member of the matrix metalloproteinases (MMPs) family, is a zinc-dependent endopeptidase involved in cardiovascular disease and cancer. MMP-2 contributes to platelet adhesion and aggregation by amplifying the platelet response to primary stim-

uli. To date, no highly selective MMP-2 inhibitors have been identified for potential use in humans.

Aims: Aim of our work was to create a panel of nanobodies highly selective for human MMP-2 and to assess their effects on platelet function, and their applicability as conjugated nanobodies for use in flow-cytometry or immunocyto/histochemistry.

Methods: We developed a library of 61 nanobodies by immunizing an Alpaca with human recombinant active MMP-2, purified from culture medium of transfected HEK293 cells. Each member of the library was tested for its effect on MMP-2 gelatinolytic activity by reverse zymography, and on platelet aggregation by Light Transmission Aggregometry (LTA). One nanobody was conjugated with fluorescein isothiocyanate (FITC) for evaluation in immunocyto/histochemistry and flow-cytometry.

Results: Three MMP-2-inhibiting nanobodies were characterized. Nanobody VHH-MMP-2-144 clone was a strong inhibitor of MMP-2 gelatinolytic activity. VHH-MMP-2-114 clone inhibited platelet aggregation induced by collagen $2 \mu\text{g mL}^{-1}$ (% inhibition = $75 \pm 11\%$) and by ADP $5 \mu\text{M}$ (% inhibition = $53 \pm 7.6\%$). VHH-MMP-2-171 blocked MMP-2 gelatinolytic activity and inhibited platelet aggregation induced by collagen $2 \mu\text{g mL}^{-1}$ (% inhibition = $30 \pm 2.9\%$) and by ADP $5 \mu\text{M}$ (% inhibition = $40 \pm 4.4\%$). FITC-conjugated VHH-MMP-2-114 ($4 \mu\text{g}$ per 100k cells) was able to highlight MMP-2 on the surface of 93% of HeLa cells, both in flow-cytometry and immunocytochemistry.

Conclusion: We have developed a panel of nanobodies against human MMP-2 and have identified 3 clones that are able to block the MMP-2 gelatinolytic activity and/or its potentiating effect on platelet aggregation. These nanobodies are a promising tool for the study of MMP-2 in cardiovascular pathophysiology.

Disclosure of Interest: None declared.

OR400

High on aspirin platelet reactivity predicts cardiac death in acute coronary syndrome patients undergoing PCI (RECLOSE2-ACS study)

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Background: High on clopidogrel platelet reactivity (HPCR) is associated with a higher risk for MACE in patients with ACS undergoing PCI. Conflicting data are available on high on aspirin platelet reactivity (HAPR) and clinical outcome.

Aims: To evaluate the possible role of HAPR in ACS pts undergoing PCI.

Methods: We performed a prospective study of 1789 patients with ACS undergoing PCI in whom platelet reactivity was assessed by light transmittance aggregometry (APACT4, Helena Laboratories, Milan, Italy) using arachidonic acid (AA) as an agonist. HAPR was found in 20.3% of patients (364/1789).

Results: Patients with HAPR were significantly older, and with a higher prevalence of hypertension, diabetes and reduced ejection fraction. Patients with three-vessel disease and multivessel PCI had a significantly higher prevalence of HAPR. In addition, total stent length, number of stents per patient and use of DES were significantly higher in HAPR patients. At a 24 month-follow-up, in 89 patients we registered a cardiac death; in 41 a myocardial infarction was documented, in 22 an ischemic stroke, in 59 a stent thrombosis. Sixteen patients underwent a urgent revascularization.

The prevalence of cardiac death was 9.7% in HAPR (35/362) and 3.8% in no-HAPR (54/1410) [HR 2.63 (1.72–4.02), $P < 0.0001$]; stent

thrombosis 6.1% in HAPR (22/362) vs. 2.6% in no-HAPR (37/1410) [HR 2.4 (1.42–4.07), $P < 0.001$], whereas there were no significant differences in the other clinical end-points. At the multivariate analysis adjusted also for HPCR, HAPR was confirmed as an independent risk factor for cardiac death [HR 1.88 (1.21–2.93), $P < 0.005$, $P < 0.0001$] and stent thrombosis [HR 1.91 (1.12–3.28), $P 0.018$]. Adding HAPR to the model including clinical and procedural risk factors, and HPCR, NRI improved significantly and was 39% for cardiac death and 34.7% for stent thrombosis.

Conclusion: HAPR was found to be an independent risk factor for cardiac death and stent thrombosis in ACS patients undergoing PCI.

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Megakaryocytes and Thrombopoiesis III

OR401

Mice lacking the inhibitory collagen receptor LAIR1 exhibit a mild thrombocytosis and hyperactive platelets

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Background: Inhibition of platelet activation is important for controlling pathological thrombosis. The leukocyte-associated immunoglobulin-like receptor-1 (LAIR1) is an immunoreceptor tyrosine-based inhibition motif (ITIM)-containing collagen receptor that inhibits signalling via the immunoreceptor tyrosine-based activation motif (ITAM)-containing collagen receptor complex GPVI-FcR γ -chain. LAIR1 is expressed on hematopoietic cells and immature megakaryocytes (MKs), but is not detectable on platelets. Although the inhibitory function of LAIR1 has been described in immune cells, its physiological role in MKs and platelets has not been explored.

Aims: We investigated the *in vivo* role of LAIR1 in MK development and platelet production.

Methods: LAIR1 knockout (KO) mice were generated. MKs and platelets were analysed using standard protocols.

Results: LAIR1 KO mice exhibit a significant increase in platelet counts, prolonged platelet half-life *in vivo*, and increased proplatelet formation *in vitro*. Platelets from LAIR1 KO mice exhibited a mild enhancement of reactivity to collagen and the GPVI-specific agonist collagen-related peptide (CRP), despite not expressing LAIR1. As a result, platelets from LAIR1 KO mice formed larger aggregates on a collagen matrix under arterial shear. Platelets from LAIR1 KO mice exhibited an increased tyrosine phosphorylation of the FcR γ -chain, the activation loop of Src Family Kinases (SFKs), tyrosine kinase Syk and its downstream target PLC γ 2 in response to CRP. Convulxin-mediated SFK, Syk and PLC γ 2 activation was also enhanced in LAIR1 KO MKs.

Conclusion: We show that LAIR1 is a negative regulator of platelet production and reactivity to collagen. Absence of LAIR1 results in increased SFK activity in MKs that gets transmitted to platelets, rendering them hyper-reactive to collagen. We show for the first time a cellular phenotype associated with LAIR1 deficiency adding to the

growing evidence that ITIM-containing receptors are critical regulators of platelet formation and function.

Disclosure of Interest: None declared.

OR402

Identification of *hox* genes controlling thrombopoiesis in zebrafish

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Background: Thrombocytes are nucleated blood cells functionally equivalent to mammalian platelets which play a central role in hemostasis. They also possess megakaryocyte features because thrombocytes and megakaryocytes have similar transcriptional machinery. *Hox* genes have already been shown to play a role in megakaryocyte development. However, a comprehensive genome-wide scan of all *hox* genes that play a role in megakaryopoiesis has not been done.

Aims: The goal of this study is to comprehensively knock-down *hox* genes to identify specific *hox* genes involved in zebrafish thrombopoiesis.

Methods: We performed flow cytometry to count thrombocytes using white cell fraction of zebrafish blood fluorescently labeled with mepacrine. For knockdown of *hox* genes we injected adult zebrafish with a control morpholino piggybacked with a standard oligonucleotide antisense to the cognate mRNA.

Results: We initially selected five *hox* genes, *hoxa10b*, *hoxb2a*, *hoxc5a*, *hoxc11b*, and *hoxd3a* from our earlier microarray analysis. We tested for the differential expression of these *hox* genes in both young and mature thrombocytes by RT-PCR. We found that *hoxc11b* was expressed only in young thrombocytes and that the remaining four genes were expressed in both young and mature thrombocytes. We then performed knockdown on all the five *hox* genes and counted thrombocytes. We found that knockdown of *hoxa10b*, *hoxb2a*, *hoxc5a*, and *hoxd3a* showed reduction in the thrombocyte counts while knockdown of *hoxc11b* increased thrombocyte counts. Using this strategy, we then screened another 47 *hox* genes in the zebrafish genome and found that knockdown of *hoxa9a* and *hoxb1a* also resulted in decreased thrombocyte number.

Conclusion: In conclusion we found six *hox* genes that are positive regulators and one *hox* gene which is a negative regulator for thrombocyte development.

Disclosure of Interest: None declared.

OR403

Proof of principle for efficiency in thrombosis of shear-generated platelets from cultured megakaryocytes

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Background: Platelets originate from megakaryocytes (MK) by intravascular fragmentation. Evidence for platelet production from human MK exposure to high shear has already been demonstrated.

Aims: To assess whether platelets produced *in vitro* after exposure of MK to high shear rates, were functional in *in vivo* models.

Methods: Microchannels were precoated with murine recombinant von Willebrand factor (VWF), and mature murine MK were perfused for 45 min at a shear rate of 1800 s⁻¹ via an electropneumatic pump through a Bioflux200 microfluidic platform. In some experiments, human MK were perfused on human VWF-coated microchannels.

Results: Murine bone marrow MK produced proplatelets and platelets during high shear exposure. This was not accompanied by platelet activation since > 90% platelets released from MK were translocating on VWF and < 5% were firmly adherent. Shear-generated platelets present hallmarks of functional platelets *in vitro* and *in vivo*. These shear-generated platelets expressed normal glycoprotein levels, responded to platelet agonists by reorganizing their cytoskeleton and by shifting the conformation of the integrin α IIb β 3 to an activated state. Using the FeCl₃ thrombosis model, these platelets specifically incorporated into the growing thrombus *in vivo*. Finally, survival of human platelets in an immunodeficient mouse model was assessed. Human shear-generated platelets infused into Rag2^{-/-} γ c^{-/-} mice were detected up to 16 h *in vivo*.

Conclusion: These data indicate the potential functionality of platelets generated *in vitro* following MK exposure to high shear rates. This constitutes a proof of principle for the efficiency in thrombosis of shear-generated platelets.

Disclosure of Interest: None declared.

OR404

A novel function of CLEC-2 in megakaryopoiesis: CLEC-2/PDPN microenvironment facilitates expansion and maturation of megakaryocytes

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Background: C-type lectin-like receptor 2 (CLEC-2) is a novel platelet activation receptor. Podoplanin (PDPN) was identified as an endogenous CLEC-2 ligand. The CLEC-2/PDPN axis plays crucial roles for the vascular/lymphatic separation during embryonic development and the integrity of high endothelial venules in lymph nodes. Interestingly, CLEC-2 knockout (KO) and megakaryocyte (MK)/platelet-specific conditional KO (cKO) mice show thrombocytopenic and mild anemic phenotypes.

Aims: The aim of this study is to explore the new role of CLEC-2 for megakaryo/thrombopoiesis.

Methods: Bone marrow (BM) cells were separated from femurs and tibias. The number and ploidy of MKs were analyzed by FACS. BM progenitor cells were isolated by lineage positive cells depletion. Expansion of MKs was examined using MegaCult-C. Maturation of MKs was estimated by proplatelet formation (PPF) ratio. To explore PDPN positive BM stromal cells, immunohistochemistry (IHC) or immunocytochemistry (ICC) was performed.

Results: The number of BM MKs was decreased in CLEC-2 cKO mice. CFU-MK assay showed that recombinant PDPN stimulation accelerated expansion of WT MK progenitors. Next, through the IHC exploration in BM sections, we found PDPN⁺ BM stromal cells which were of immunophenotypes similar to fibroblastic reticular cells (FRCs) in lymph nodes. We also observed that subsets of MK colonies located in the vicinity of these PDPN⁺ cells. In co-culture with MKs and BM FRCs, we observed that expansion of CLEC-2 WT MK progenitors, but not that of cKO MK progenitors, was drastically accelerated. In addition, co-culture with MKs and BM FRCs promoted PPF of CLEC-2 WT MKs, but not that of cKO MKs.

Conclusion: The thrombocytopenic phenotype in CLEC-2 cKO mice could be attributed to the lack of CLEC-2/PDPN axis between MKs and BM FRCs. These results have led us to a hypothesis that expansion and maturation of MK lineages are partly supported by CLEC-2/PDPN microenvironment consisting of BM FRCs.

Disclosure of Interest: None declared.

OR405

The dense blood vessel network in the murine bone marrow spatially limits megakaryocyte migration

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Background: Megakaryocytes (MKs) are derived from megakaryocyte progenitors and mature under the influence of thrombopoietin, a process termed megakaryopoiesis. MK development is thought to progress spatially from the endosteal niche to the sinusoidal vascular niche where terminal maturation and thrombopoiesis occur. However, the location of MK maturation and the functional significance of MK migration towards the vascular niche for thrombopoiesis remain ill-defined. Previous studies on megakaryopoiesis were hampered by the need to section bones, possibly resulting in cutting artefacts and an underestimation of MK-to-vessel contacts and MK volumes. Therefore, we developed a new method to visualize MKs in their 3D environment using light-sheet fluorescence microscopy (LSFM), which enables imaging of large tissue samples.

Aims: We aimed to visualize the spatio-temporal distribution and responses of MKs in the intact murine bone marrow during acute thrombocytopenia.

Methods: Megakaryopoiesis was induced by depleting circulating platelets with a bolus injection of anti-GPIIb antibodies. Sterna were antibody-stained for MKs and endothelial cells, paraformaldehyde-fixed, chemically cleared and imaged by LSFM.

Results: We established a method for visualizing MKs in the intact bone, at a resolution that enables the visualization of proplatelets. In addition, thrombocytopenia was induced and the effects on megakaryopoiesis were monitored for several days. This approach allowed us to quantify changes in MK volume, localization and number in the bone marrow of the unsectioned sternum. Interestingly, we could identify an extremely dense network of blood vessels in the bone marrow, revealing an unexpected spatial limitation for MK migration.

Conclusion: This novel method complements current *in situ* and *ex vivo* techniques. The high quality 3D data challenge the current concept of MK migration and give a more detailed insight into the environmental regulation of MKs and the functional significance of MK migration for thrombopoiesis.

Disclosure of Interest: None declared.

Von Willebrand factor – II

OR406

Single nucleotide variants C.2365A>G and C.2385T>C are associated with increased VWF plasma levels through an effect on mRNA half-life

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Background: von Willebrand factor (VWF) plasma levels vary considerably; between 50 and 200 IU dL⁻¹ in 95% of the general population. Several factors have been associated with this variation including ABO blood group and VWF single nucleotide variants (SNV). Several studies highlighted significant association between SNV rs1063856

(c.2365A>G; p.Thr789Ala) and rs1063857 (c.2385T>C; p.Tyr795=) with VWF level.

Aims: To further investigate the association between SNV rs1063856 and rs1063857 with VWF level and elucidate the mechanism(s) involved.

Methods: SNV were genotyped in healthy controls (HC) recruited by the MCMDM-1VWD study. *In vitro* expression of VWF containing SNV was performed in HEK293T cells, followed by measurement of VWF mRNA expression using TaqMan quantitation and VWF:Ag via ELISA. mRNA half-life was measured after inhibiting transcription using 5 µg mL⁻¹ Actinomycin D at 0, 2, 3 and 4 h post treatment.

Results: Genotype analysis of HC showed that both variants were significantly associated with increased VWF level (c.2365A>G AA: 96.4 IU dL⁻¹; AG: 99.6 IU dL⁻¹; GG: 114.3 IU dL⁻¹; *P* < 0.0001; *n* = 1095; c.2385T>C TT: 96.4 IU dL⁻¹; TC: 99.5 IU dL⁻¹; CC: 113.9 IU dL⁻¹; *P* < 0.0001; *n* = 1109) and are in strong LD (*r* = 99.01%). *In vitro* expression (*n* = 3) showed similar results (c.2365A>G AA: 100%; AG: 115%; GG: 131%; *P* = 0.0009; c.2385T>C TT: 100%; TC: 104%; CC: 128%; *P* = 0.006; both SNV *in trans* WT: 100%; heterozygous: 113%; homozygous: 125%; *P* = 0.03). mRNA expression (*n* = 3) was also increased (c.2365A>G AA: 100%; AG: 144%; GG: 153%; *p*0.005; c.2385T>C TT: 100%; TC: 134%; CC: 153%; *P* = 0.02; both SNV WT: 100%; heterozygous: 156%; homozygous: 174%; *P* < 0.001). RNA half-life (*n* = 1) increased by 18.3% for c.2365A>G, 16.5% for c.2385T>C and 18.9% for both SNV *in trans*.

Conclusion: SNV c.2365A>G and c.2385T>C are associated with increased VWF mRNA and protein expression levels. This observed difference appears to be due to a longer mRNA half-life for the non-reference allele.

Disclosure of Interest: None declared.

OR407

Type 2B von Willebrand disease: the biological consequences of a prolonged embrace

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Background: Type 2B mutations cluster near the termini of VWF-A1 domain where they support either an energetic contribution that augments binding or reduce a steric clash that impedes interactions with GPIIb α . Such mutations can also prolong the GPIIb α -VWF-A1 bond lifetime. However, it is unclear whether an alteration in bond lifetime contributes to the defect in hemostasis associated with type 2B VWD.

Aims: To dissect the mechanism(s) by which the 2B mutation I1309V disrupts hemostasis and thrombosis by studying the biophysical properties of mutant murine GPIIb α -VWF-A1 complexes and generating mice with mutant A1 domains.

Methods: Bond lifetime was measured by high temporal resolution video microscopy. Mice were generated with the following VWF-A1 mutations: (i) I1309V (minor binding site); (ii) R1326H (major binding site) that supports human but not mouse GPIIb α binding; (iii) I1309V and R1326H. Platelet-VWF interactions were assessed by LTA, flow chamber, tail bleeding, and laser-induced arterial injury.

Results: I1309V prolonged, R1326H shortened, and the combined mutations yielded a GPIIb α -VWF-A1 bond lifetime comparable to the native murine complex. The I1309V/R1326H substitutions did, however, augment interactions with human GPIIb α . In mice, I1309V produced a type 2B phenotype, with loss of HMW multimers and defects in hemostasis and thrombosis. In contrast, the R1326H impaired platelet-VWF interactions, yielding a phenotype similar to VWF null mice. In the context of R1326H, however, the I1309V could no longer

induce type 2B VWD: hemostatic/thrombotic properties of VWF possessing the I1309V/R1326H double mutations were indistinguishable from the native protein. However, infusion of human platelets resulted in a type 2B like phenotype.

Conclusion: (i) I1309V mutation yields a type 2B phenotype only if it can prolong the GPIIb-IIIa-VWF-A1 lifetime beyond that of the native complex; (ii) lifetime of this interaction must be maintained within a specific range to support effective hemostasis.

Disclosure of Interest: None declared.

OR408

Pathological VWF fibers resist tPA and ADAMTS13 while facilitating the contact pathway and shear-induced platelet activation

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Background: Under severe stenotic conditions seen in pathologic coronary vessels, von Willebrand Factor (vWF) in plasma multimerizes into large insoluble fibers at pathological shear rates. Due to the importance of high shear stenotic anatomies in many cardiovascular pathologies, it is essential to understand the behavior and properties of high molecular weight, fibrous vWF and its interactions with platelets.

Aims: Implement a micropost-impingement microfluidic device to multimerize and capture fibrous vWF in order to examine the thrombolytic resistance of vWF fibers, procoagulant properties, and role in platelet activation.

Methods: A stenosis-shaped microfluidic device with a micropost in flow was used to shear and capture fibrous vWF from platelet-free plasma (PFP).

Results: Captured vWF fiber thickness increased in a shear-dependent manner between 5000 s^{-1} and $10,000\text{ s}^{-1}$. Stress-strain tests in flow estimated the elastic modulus of fibrous vWF to be $\sim 50\text{ MPa}$. Insoluble vWF fibers were not amyloid since they rapidly dissolved in trypsin or 2% SDS, but they were resistant to 50 nM ADAMTS13 or 100 nM tPA in plasma. Following fiber formation, low corn trypsin inhibitor (CTI)-treated ($4\text{ }\mu\text{g mL}^{-1}$) recalcified citrated plasma perfusion at 1500 s^{-1} resulted in local fibrin formation on the vWF fibers, a result not observed with purified type 1 collagen or a naked micropost. Since fibrin formation did not occur on vWF fiber formed using EDTA/PPACK/Apiaban/high CTI-treated PFP, it is thought that contact pathway factors accumulated in the vWF fibers as they multimerized. When PPACK-inhibited whole blood was perfused over vWF fibers, platelets rolled and arrested on the surface of vWF, but only displayed P-selectin at regions of pathological shear. Platelet arrest on vWF fibers was blocked with the $\alpha_{\text{IIb}\beta_3}$ antagonist GR144053.

Conclusion: These observations reveal vWF fiber-contact pathway crosstalk during acute myocardial infarction and define a new mechanism of resistance of occlusive clots to thrombolytic therapy.

Disclosure of Interest: None declared.

OR409

The scavenger receptor stabilin-2 (STAB-2) mediates clearance of human von Willebrand factor and factor VIII by liver sinusoidal endothelial cells

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Background: Plasma levels of von Willebrand factor (VWF) and factor VIII (FVIII) influence the risk for bleeding and thrombosis. The mechanisms by which VWF and FVIII are cleared from the plasma are largely uncharacterized. Polymorphisms in stabilin-2 (STAB-2), a scavenger receptor expressed by liver sinusoidal endothelial cells (LSECs), were associated with plasma levels of VWF and FVIII in a GWAS meta-analysis.

Aims: We investigated the ability of LSECs and STAB-2 to bind and endocytose VWF and FVIII and regulate VWF-FVIII clearance.

Methods: Internalization of VWF and FVIII by LSECs was evaluated *in vivo* using immunohistochemistry and *ex vivo* using isolated murine LSECs by immunofluorescence. The clearance of VWF and FVIII by STAB-2 was evaluated in VWF^{-/-}/STAB-2^{-/-} mice. Endocytosis of VWF and FVIII by STAB-2 was observed using transfected HEK 293 cells.

Results: Human plasma derived (pd) and murine recombinant (r) VWF infused into VWF KO mice was internalized by CD31 positive cells (Kupffer cells and LSECs). Depletion of murine LSECs with cyclophosphamide increased the half-life of infused human pdVWF (1.8-fold, $P = 0.009$). Isolated LSECs expressing CD31 and STAB-2 bound and internalized human pdVWF, and murine recombinant (r) VWF.

The half-life of infused human pdVWF was significantly longer in VWF^{-/-}/STAB-2^{-/-} mice than in VWF^{-/-} controls (VWF-FVIII complex: 2.3-fold, $P = 0.0003$; VWF (FVIII-free): 2-fold, $P = 0.0005$). The half-life of human pdFVIII bound to VWF was significantly longer in VWF^{-/-}/STAB-2^{-/-} mice (slow half-life = 1.2 fold, $P = 0.002$). STAB-2 deficiency did not influence endogenous VWF levels or the half-life of murine rVWF or pdVWF. Human and murine STAB-2 expressing HEK 293 cells bound and internalized human rVWF and pdVWF-FVIII complex, but interacted weakly with murine rVWF.

Conclusion: LSECs mediate clearance of VWF and FVIII in a VWF-dependent manner. STAB-2 is a novel clearance receptor for human VWF-FVIII complex.

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OR410

von Willebrand factor deficiency causes increased angiogenesis following ischemic tissue damage

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Background: Von Willebrand Factor (VWF) is multifunctional protein synthesised by endothelial cells. Its deficiency or dysfunction causes

Von Willebrand disease (VWD), the most common congenital bleeding disorder. VWD is associated with vascular malformations (angiodysplasia) which may lead to severe gastrointestinal bleeding. We have shown that VWF regulates angiogenesis, providing a possible mechanistic link to angiodysplasia.

Aims: To characterise the angiogenic defect in VWF^{-/-} mice during physiological and pathological angiogenesis, and investigate the molecular mechanism.

Methods: Signalling pathways were investigated in human umbilical vein endothelial cells (HUVEC). Physiological angiogenesis was studied using the mouse post-natal retina. Ischaemic induced angiogenesis was studied with a murine model of hindlimb ischaemia, induced by femoral artery ligation.

Results: In HUVEC, inhibition of VWF expression by siRNAs resulted in increased VEGF Receptor (VEGFR) 2-dependent signalling to ERK1/2 ($P < 0.001$) leading to increase proliferation; this was enhanced by cell adhesion, suggesting involvement of integrin $\beta 3$. *In vivo*, VEGFR2 and integrin $\beta 3$ are of central importance in sprouting angiogenesis in the mouse retina during development. VWF was expressed throughout retinal vasculature, with levels increasing towards the angiogenic front ($P < 0.01$), implicating VWF in the regulation of angiogenic sprouting. Supporting this model, retinas from VWF^{-/-} mice showed increased vascular density ($P < 0.05$) and tip cell numbers at the angiogenic front ($P < 0.001$). Hypoxia is a major initiator of VEGF-dependent angiogenesis. Hindlimb ischaemia caused increased tissue damage ($P < 0.05$) and angiogenesis ($P < 0.001$) in VWF^{-/-} mice, supporting a role for VWF in the inhibition of hypoxia-induced pathological angiogenesis.

Conclusion: These data indicate that a deficiency in VWF promotes physiological and pathological-induced angiogenesis through pathways involving VEGFR2.

Disclosure of Interest: None declared.

Factor VIII – III

OR411

Cell-specific FVIII expression in hemophilia A gene therapy to overcome immunological responses

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Background: Hemophilia A (HA) is a X-linked bleeding disorder due to mutations in clotting factor (F) VIII gene. HA patients are treated with recombinant or plasma-derived FVIII with high probability to develop inhibitors. Despite the efforts aimed to cure HA the immune response is still the main issue. Antigen-presentation occurring in the liver by sinusoidal endothelial cells (LSEC) or Kupffer cells (KC) induces tolerance rather than immunity to antigens locally presented to T-cells.

Aims: To study the role of LSEC and KC in HA gene transfer using lentiviral vectors (LV)-expressing FVIII driven by cell-specific promoters $\pm$ miRNA target sequences (miRTs).

Methods: We prepared LV containing GFP or hFVIII cDNA under the control of PGK (ubiquitous), CD11b (monocyte/macrophage marker) and VEC (endothelial marker) promoter alone or with several combination of miRTs, i.e. miRT142.3p (silencing in hemopoietic cells), miRT126 (silencing in endothelial cells) or miRT122 (silencing in hepatocytes), and injected HA mice.

Results: After LV characterization *in vivo* using GFP as transgene, we injected HA mice with LV.PGK.FVIII $\pm$ 142, LV.CD11b.FVIII $\pm$ 126 and LV.VEC.FVIII $\pm$ 122–142. In the group of LV.PGK anti-FVIII antibodies were detected 2w after injection and the presence of miRT142 halved the inhibitor titer. Mice injected with LV.CD11b.FVIII-126 and LV.VEC.FVIII-122-142 reached long-term phenotypic correction up to 1 year with an average of 5–6% FVIII

and absence of anti-FVIII antibodies. PCR on genomic DNA demonstrated LV integration and RT-PCR and tissue staining demonstrated hFVIII expression in desired cell targets. Moreover, in these mice tolerance was obtained since we obtained FVIII activity in FVIII-immunized mice producing anti-FVIII antibodies.

Conclusion: LV expressing FVIII under control of cell-specific promoters combined with miRT-combinations were able to overcome FVIII off-target expression limiting immune responses and providing phenotypic correction in treated HA mice.

Disclosure of Interest: None declared.

OR412

Influence of VWF-FVIII binding on arterial thrombus formation and stability in A murine model of severe type 2N VWD

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Background: von Willebrand factor (VWF) and factor VIII (FVIII) circulate in the plasma as a non-covalent complex. VWF may influence plasma FVIII activity by stabilizing FVIII levels and transporting FVIII to the site of platelet thrombus formation. Type 2N von Willebrand disease (VWD) involves impaired binding of VWF to FVIII.

Aims: The influence of VWF-FVIII binding on thrombus formation and stability was investigated in a murine model of severe type 2N VWD.

Methods: Severe type 2N VWD (R816W) mice were generated by hydrodynamic injection of murine VWF cDNA into VWF^{-/-} mice (VWF:Ag = 2 U mL⁻¹). Intravital arteriole thrombus formation in the murine cremaster was induced by hematorporphyrin.

Results: Hydrodynamic expression of wild-type (WT) VWF stabilized plasma FVIII (FVIII:C = 100%) while R816W VWF did not (FVIII:C = 20%). Time to vessel occlusion was slightly increased for R816W VWF relative to WT (1.3-fold, $P = 0.2$). Thrombus volume (1.6-fold, $P = 0.008$), average thrombus size (1.4-fold, $P = 0.0007$) and number of thrombi (1.5-fold, $P = 0.0001$) were increased for mice expressing WT VWF. R816W VWF was associated with a greater frequency of small embolic events (1.51-fold, $P = 0.05$).

To assess VWF transportation of FVIII to the thrombus, plasma FVIII stabilization was controlled by IV injection of VWF (FVIII-free) into VWF^{-/-} mice (FVIII:C = 20%). For mice infused with R816W VWF, time to occlusion was slightly delayed relative to mice infused with WT VWF (1.3-fold, $P = 0.26$). Thrombus volume (1.65-fold, $P = 0.0008$), and number of thrombi (1.3-fold, $P = 0.01$) were increased for WT relative to R816W VWF-infused mice, however average thrombus size and frequency of embolization were not significantly different.

Conclusion: Stabilization of plasma FVIII by VWF resulted in formation of larger, more stable thrombi. Impaired binding of VWF to FVIII decreased total number of thrombi formed. Patients with type 2N VWD may have normal primary hemostasis but decreased stability of formed thrombi.

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OR413

A FVIII/VWF chimeric protein with VWF independent pharmacokinetic properties

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Background: Recombinant FVIII-XTEN/VWF heterodimer is a FVIII/VWF chimeric protein which does not associate with endogenous Von Willebrand Factor (VWF) and has demonstrated comparable potency and 4-fold prolongation on half-life and efficacy compared to rFVIII in HemA mice. The half-life of rFVIII-XTEN/VWF is expected to be independent from the amount and FVIII binding affinity of circulating VWF. This unique property of rFVIII-XTEN/VWF may result in a more uniform pharmacokinetic profile in Haemophilia A patients and makes it a potential candidate for the treatment of bleeding disorders associated with low level of FVIII in patients with Von Willebrand disease (VWD).

Aims: To determine the pharmacokinetics (PK) and pharmacodynamics of rFVIII-XTEN/VWF in FVIII deficient (HemA) and FVIII/VWF deficient (DKO) mice.

Methods: rFVIII-XTEN/VWF heterodimer comprises a FVIII-XTEN-Fc chain covalently associated with a VWF D'D3-XTEN-Fc chain via the two Fc-domains. Activity PK profiles were determined in HemA and DKO mice in comparison to rFVIII; prolonged efficacy was evaluated by ex vivo ROTEM.

Results: The half-life of rFVIII is dramatically decreased from 8 h in HemA mice to 15 min in DKO mice; in contrast, the half-life of rFVIII-XTEN/VWF is similar in HemA and DKO mice at 34 and 35 h respectively. In addition, similar MRT, Clearance and AUC were also observed for rFVIII-XTEN/VWF in the two mouse strains. Correlated to the extended half-life, rFVIII-XTEN/VWF also demonstrated at least 30-fold extension on procoagulant activity compared to rFVIII in DKO mice as measured by ex vivo ROTEM.

Conclusion: The PK profile of rFVIII-XTEN/VWF heterodimer is independent from the circulating VWF, which may lead to a more uniform dosing regimen if used in HemA patients. It has also demonstrated significantly extended procoagulant activity compared to rFVIII in VWF deficient animals, thus could potentially be a good candidate as prophylactic treatment for some types of VWD.

Disclosure of Interest: S. Patarroyo-White Shareholder of: Biogen Idec, Employee of: Biogen Idec, D. Drager Shareholder of: Biogen Idec, Employee of: Biogen Idec, T. Liu Shareholder of: Biogen Idec, Employee of: Biogen Idec, A. Ismail Shareholder of: Biogen Idec, Employee of: Biogen Idec, J. Liu Shareholder of: Biogen Idec, Employee of: Biogen Idec, A. Holthaus Shareholder of: Biogen Idec, Employee of: Biogen Idec, E. Chhabra Shareholder of: Biogen Idec, Employee of: Biogen Idec, J. Kulman Shareholder of: Biogen Idec, Employee of: Biogen Idec, V. Schellenberger: None declared, R. Peters Shareholder of: Biogen Idec, Employee of: Biogen Idec.

OR414

FVIII-VWF interaction assessed by hydrogen-deuterium exchange mass spectrometry and binding affinity

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Background: FVIII binding site on VWF has been localised to the D'D3 domains,¹ with the N-terminus of D' being essential for high

affinity binding.¹¹ The D3 domain can be divided into the subdomains VWD3, C8-3, TIL-3 and E-3.¹¹¹

Aims: To structurally investigate the interaction between human blood-coagulation factor VIII and von Willebrand factor.

Methods: Monomeric VWF fragments were expressed in CHO cells, containing C1099S/C1142S substitutions to prevent dimerization. The binding of rFVIII (turoctocog alfa, NovoEight®) to VWF fragments was characterised for affinity by competitive ELISA and for structural dynamics and interface mapping by hydrogen-deuterium exchange mass spectrometry (HDXMS).

Results: The IC50 values for rFVIII binding to VWF fragments were 10–15 nM for D'D3A1 and D'D3 and 2 µM for D'VWD3 and D'. In comparison, the IC50 for rFVIII binding to plasma derived VWF was 1 nM.

The HDXMS analysis revealed several regions in rFVIII light chain (LC) with lowered deuterium incorporation upon binding to D'D3A1, i.e., of residues within a3 1671–1680; A3 1742–1751 and 1856–1874; C1 2061–2074 and 2125–2148; C2 2260–2280. Identical observations were found within the A3 and C1 domains upon D'D3, D'VWD3, and D' binding. In contrast, discrepancies for the residues 2285–2300 in the C2 domain were observed upon binding to D'VWD3 and D'. HDXMS analysis of rFVIII binding to plasma derived VWF in hypertonic solution (0.9 M NaCl) was comparable to rFVIII binding to D'D3A1.

Conclusion: The study shows that nM range binding affinity of rFVIII to VWF required the full D'D3 domain. Overall good structural agreement was found for the HDXMS analysis of rFVIII binding to VWF fragments. However, structural discrepancies were indicated of the FVIII C2 domain dependent on the length of the VWF fragments and in agreement with binding affinity variations. These findings suggest a complex binding of FVIII to VWF inducing conformational changes of FVIII LC.

¹JBC 1987, 262, 8443

¹¹JBC 2013, 288, 393

¹¹¹Blood 2012, 120, 449

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OR415

Improved pharmacokinetics and *in vivo* efficacy of platelet-targeted factor VIIa-XTEN in human alpha IIb transgenic/hemophilia A mice

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Background: Potent recombinant activated Factor VII (rFVIIa) variants with prolonged pharmacokinetic properties may allow for effective prophylaxis treatment for hemophilia patients with inhibitors and provide improved treatment and protection from severe joint damage. We combined recombinant XTEN and platelet targeting technologies to improve both the pharmacokinetics and the activity of FVIIa. We have previously shown that fusion of the XTEN-polypeptide increases the hydrodynamic radius of rFVIIa, improving its pharmacokinetic properties, and that the clot formation activity of rFVIIa is significantly improved by targeting it to platelets, without affecting platelet activity. The latter is achieved by the recombinant fusion of an antibody fragment, which binds the platelet receptor human alpha IIb.

Aims: To evaluate the pharmacokinetics and efficacy of platelet-targeted rFVIIa-XTEN proteins in humanized alpha IIb transgenic/hemophilia A mice (Tg/HemA).

Methods: We used Tg/HemA mice to assess the pharmacokinetics of platelet-targeted rFVIIa-XTEN by both plasma activity and ELISA. Clotting activity was determined by *ex-vivo* ROTEM assays and the efficacy to stop bleeds was explored by tail-clip experiments.

Results: Our results show that platelet-targeted rFVIIa-XTEN has a longer half-life in Tg/HemA mice compared to rFVIIa based on plasma activity levels. ROTEM assays, using blood from dosed mice, showed greatly enhanced *ex-vivo* clotting activity compared to rFVIIa at various doses and over time, suggesting prolonged efficacy. Finally, tail-clip experiments demonstrated improved efficacy in stopping bleeds for platelet-targeted rFVIIa-XTEN.

Conclusion: Platelet-targeted rFVIIa-XTEN fusion proteins show, compared to rFVIIa in Tg/HemA mice, improved efficacy in acute tail clip experiments, improved ROTEM potency and decreased clearance; setting the basis for a potential prophylaxis treatment option for hemophilia patients with inhibitors.

Disclosure of Interest: A. Van Der Flier Shareholder of: Biogen Idec, Employee of: Biogen Idec, J. Salas Shareholder of: Biogen Idec, Employee of: Biogen Idec, Z. Liu Shareholder of: Biogen Idec, Employee of: Biogen Idec, T. Ashworth Shareholder of: Biogen Idec, Employee of: Biogen Idec, E. Kistanova Shareholder of: Biogen Idec, Employee of: Biogen Idec, S. Smith Shareholder of: Biogen Idec, Employee of: Biogen Idec, K. Chen Shareholder of: Biogen Idec, Employee of: Biogen Idec, D. Rabinovich Shareholder of: Biogen Idec, Employee of: Biogen Idec, S. Tan Shareholder of: Biogen Idec, Employee of: Biogen Idec, V. Schellenberger Shareholder of: Amunix, Employee of: Amunix, D. Light Shareholder of: Biogen Idec, Employee of: Biogen Idec, R. Peters Shareholder of: Biogen Idec, Employee of: Biogen Idec.

Platelets – III

OR416

Targeted downregulation of CLEC-2 occurs through SRC family kinase dependent internalization in mouse platelets

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Background: The C-type lectin-like receptor 2 (CLEC-2) plays an important role in thrombosis, hemostasis and prevention of inflammatory bleeding. Ligation of CLEC-2 induces phosphorylation of the hemITAM by Src family kinases (SFK) and Syk, initiating a signaling cascade which culminates in the activation of phospholipase C gamma 2. We have previously shown that treatment of mice with the CLEC-2 specific antibody INU1 results in depletion of CLEC-2 in circulating platelets, which is preceded by a severe transient thrombocytopenia. Such CLEC-2 depleted mice are protected from arterial thrombosis, but do not show a major bleeding defect. The mechanisms underlying this targeted CLEC-2 downregulation are unknown.

Aims: We aimed to identify the mechanisms underlying INU1-induced thrombocytopenia and receptor depletion *in vivo* by utilizing pharma-

cological inhibitors and mouse lines deficient in critical signaling molecules.

Methods: For depletion of CLEC-2 in circulating platelets, mice were treated with the antibody INU1 (100 µg i.v.). Platelet counts and function were analyzed *ex vivo* for up to 10 days using flow cytometry, biochemical methods and functional assays.

Results: INU1-treatment induced a severe transient thrombocytopenia in wild-type, but not CLEC-2 deficient mice, independently of FcRγ-mediated phagocytosis or integrin-mediated platelet aggregation. In contrast, INU1-induced thrombocytopenia was strongly attenuated in mice with a platelet-specific Syk deficiency, while CLEC-2 downregulation was fully preserved. Further studies revealed that INU1-induced CLEC-2 downregulation occurred through internalization *in vitro* and *in vivo* and was completely blocked by SFK inhibitors, as was the associated thrombocytopenia.

Conclusion: Our results show for the first time that CLEC-2 can be downregulated from the platelet surface through SFK-dependent internalization, and that this can be uncoupled from the undesired antibody-induced thrombocytopenia thereby increasing its potential clinical use.

Disclosure of Interest: None declared.

OR417

Protein disulfide isomerase denitrosylase activity in platelet activation

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Background: A role for PDI in thrombus formation is now established and PDI antagonists are in clinical trials. Yet the mechanisms by which PDI contributes to thrombus formation are unknown.

Aims: To determine whether the denitrosylase activity of PDI contributes to its role in platelet activation and thrombus formation.

Methods: We developed a series of PDI active site mutants and screened for mutants with increased denitrosylase activity (denitrosylase-biased mutants) or increased reductase activity (reductase-biased mutants).

Results: Screening PDI active site mutants identified a reductase-biased and a denitrosylase-biased mutant. Using differential cysteine alkylation followed by mass spectroscopy to determine the redox potential of active site cysteines, we showed that oxidase-biased mutant had the highest redox potentials ($E^{\circ} = -205$ mV), while the denitrosylase-biased mutant had the lowest redox potentials ($E^{\circ} = -268$ mV). Incubation of platelets with a nitric oxide (NO) donor resulted in total inhibition of platelet activation by multiple agonists. PDI reversed NO-induced platelet inhibition, while an enzymatically inert mutant had no effect. The denitrosylase-biased mutant was even more effective than native PDI in reversing NO-induced inhibition. We next evaluated the effect of PDI on nitrosylation of a2bb3. NO exposure resulted in a2bb3 nitrosylation. Subsequent exposure to PDI resulted in denitrosylation of a2bb3. In thrombus formation studies performed *in vivo*, GSNOR^{-/-} mice lacking GSNO reductase, an enzyme critical for metabolism of GSNO (the major physiologic NO donor), demonstrated impaired platelet accumulation and fibrin formation. PDI reversed this inhibition, while inactive PDI did not.

Conclusion: These studies demonstrate that redox potential is a critical determinant of PDI reductase vs. denitrosylase activity and show that PDI denitrosylase activity functions in platelet activation and, potentially, in thrombus formation.

Disclosure of Interest: None declared.

OR418

PEAR1: a novel link between ige-mediated allergy and cardiovascular diseaseSun Y¹, Vandenbriele C², Kauskot A², Verhamme P², Hoylaerts MF² and Wright GJ¹¹Cell Surface Signalling Laboratory, Wellcome Trust Sanger Institute, Cambridge, UK; ²Center for Molecular and Vascular Biology, Department of Cardiovascular Sciences, KU Leuven, Leuven, Belgium**Background:** Genome-wide association studies for platelet function and cardiovascular disease repeatedly identified polymorphisms linked to Platelet endothelium aggregation receptor 1 (PEAR1), an 'orphan' cell surface receptor involved in stabilizing platelet aggregates.**Aims:** We sought to identify the extracellular ligand(s) of PEAR1.**Methods:** We created a protein microarray representing the secretome and receptor repertoire of the human platelet.**Results:** One hundred and seventy-three Recombinant proteins were expressed. Using an avid soluble recombinant PEAR1 protein and a systematic screening assay designed to detect extracellular interactions, we identified the high-affinity IgE-binding subunit FcεR1α, as a PEAR1 ligand. FcεR1α and PEAR1 interacted with a strong affinity ($K_D \sim 30$ nM). Pre-complexing FcεR1α with IgE potently inhibited the FcεR1α-PEAR1 interaction. Oligomerised FcεR1α potentiated platelet aggregation and led to PEAR1-phosphorylation, an effect that was inhibited by IgE. This (shows how a protein microarray resource can be used to gain important insight into the function of platelet receptors, and) provides a mechanistic basis for the initiation of PEAR1 signaling in aggregation. The identification of FcεR1α as an activating ligand for PEAR1 and the finding that IgE can inhibit this interaction suggests a link between IgE and platelet function. The *in vivo* reduction of circulating IgE by a clinical anti-IgE monoclonal antibody omalizumab, preventing IgE binding to FcεR1α, showed that omalizumab can relieve IgE-mediated inhibition of the FcεR1α-PEAR1 interaction and may provide an explanation for the increased risk of cardiovascular disease associated with omalizumab use.**Conclusion:** We developed a platelet protein microarray resource to gain new insights into the function of platelet receptors. We identified, the FcεR1α-PEAR1 interaction and its regulation by endogenous IgE, which provides a mechanism to explain previously under-appreciated interactions between allergy and cardiovascular disease.**Disclosure of Interest:** None declared.

OR419

Characterization of a novel integrin binding protein that is essential for alphallbbeta3 outside-in signaling and hemostasisXiang B¹, Zhang G¹, Ye S¹, Huang C¹, Liu J², Tao M³, Ruan C⁴, Smyth S¹, Whiteheart S¹ and Li Z¹¹University of Kentucky, Lexington; ²Mayo Clinic in Arizona, Scottsdale, USA; ³The First Affiliated Hospital of Soochow University; ⁴Jiangsu Institute of Hematology, Suzhou, China**Background:** Integrins are heterodimeric (α/β) membrane proteins that play fundamental roles in many biological processes, e.g. cell adhesion and spreading, which are important for platelet function and hemostasis. Integrin function is modulated by bi-directional transmembrane signaling: inside-out and outside-in, which is mediated through the interactions between integrin cytoplasmic tails and intracellular, regulatory proteins.**Aims:** To identify novel integrin binding proteins and gain insights into α IIb β 3 function.**Methods:** Mass spectrometry, GST-Pull down assay, confocal microscopy, and co-immunoprecipitation were used for searching and con-

firming the integrin binding proteins. Recombinant integrin activation model in CHO cells and megakaryocyte- and platelet-specific conditional knockout mice were used to investigate the function of the integrin binding proteins.

Results: We identified VPS33B, a member of the Sec1/Munc18 (SM) family and component of the CORVET/HOPS sorting complexes, as a direct binding protein of the integrin β subunits. We show that overexpression of VPS33B in CHO cells potentiated α IIb β 3 outside-in signaling but not inside-out signaling. Platelets, from megakaryocyte- and platelet-specific VPS33B conditional knockout mice we generated, had normal morphology yet their spreading on fibrinogen was impaired and they failed to support clot retraction. Platelet aggregation and ATP secretion in response to low-dose thrombin were reduced in the VPS33B knockout mice. α IIb β 3-mediated endocytosis of fibrinogen was also defective. Tail bleeding times were prolonged in the VPS33B knockout mice. Furthermore, VPS33B acted upstream of the RhoA-ROCK-MLC and Rac1 dependent pathways that leads to clot retraction and cell spreading, respectively.**Conclusion:** Our work demonstrates that VPS33B plays an important role in hemostasis and that vesicular trafficking complexes are a novel class of modifiers of integrin function.**Disclosure of Interest:** None declared.

OR420

GPVI interaction with polymerized fibrin boosts thrombin generation and thrombus growthMangin P^{1,2,3}, Mammadova-Bach E^{1,2,3}, Dumont B^{4,5,6}, Loyau S^{4,5,6}, Gachet C^{1,2,3}, Olivier V^{4,5,6} and Jandrot-Perrus M^{4,5,6}¹Université de Strasbourg; ²Fédération de Médecine Translationnelle de Strasbourg (FMTS); ³UMR_S949, Inserm, Université de Strasbourg, Etablissement Français du Sang-Alsace (EFS-Alsace), Strasbourg; ⁴UMR_S1148, Inserm; ⁵Université Paris-Diderot; ⁶CHU Bichat, Paris, France**Background:** Fibrin, the end product of the coagulation cascade, consolidates the platelet plug at site of vascular injury. Polymerized fibrin supports platelet adhesion and aggregation through integrin α IIb β 3, GPIb in the presence of thrombin, and additional yet unknown platelet receptor(s).**Aims:** To evaluate whether glycoprotein VI (GPVI) could be a functional fibrin receptor.**Methods:** Thrombin generation was measured using a calibrated automated thrombogram. GPVI was blocked using the Fab of the monoclonal antibody 9O12. GPVI binding to fibrin was measured *in vitro* using recombinant soluble GPVI (GPVI-Fc). Flow based adhesion assays were performed in capillary chambers coated with polymerized fibrin.**Results:** Thrombin generation triggered by tissue factor, kaolin or low doses of thrombin was impaired in the PRP of GPVI-deficient patients or Fab 9O12-treated PRP. In a purified system, fibrinogen dose-dependently increased the thrombin peak, with a blunting effect of Fab 9O12. Blocking fibrin polymerization with GPRP reduced the thrombin peak in normal PRP, but not in Fab 9O12-treated PRP and the PRP of GPVI-deficient patients. Binding assays showed a dose-dependent and specific (blocked by the Fab 9O12) binding of GPVI-Fc to fibrin polymers. Platelets adhered to polymerized fibrin resulting in platelet shape change and exposure of phosphatidylserine. Platelet adhesion to a fibrin network was reduced with Fab 9O12 or when GPVI was deficient at both low (300 s⁻¹) and high (1500 s⁻¹) wall shear rates. Fibrin interaction with GPVI and α IIb β 3 were not mutually exclusive. Finally when hirudinized blood was perfused at a shear rate of 1500 s⁻¹ onto preformed fibrin-rich clots, the Fab 9O12 decreased the recruitment of platelets by up to 93%.

Conclusion: Here we show for the first time that GPVI acts as a platelet receptor for polymerized fibrin with two major functions: GPVI interaction with polymerized fibrin triggers (i) a new loop amplifying thrombin generation and (ii) platelet recruitment at the clot surface.

Disclosure of Interest: None declared.

Regulation of coagulation and fibrinolysis

OR421

Plasminogen activator inhibitor-1 partially protects mice against cardiac fibrosis by inhibiting urokinase-type plasminogen activator-mediated plasminogen activation

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Background: Cardiac fibrosis, a stiffening of the cardiac tissue with resultant diminishment of heart function, leads to a variety of heart diseases. Plasminogen activator inhibitor-1 (PAI-1) is known to protect mice against cardiac fibrosis. It is speculated that PAI-1 may regulate cardiac fibrosis by inactivating urokinase-type plasminogen activator (uPA) and ultimately plasmin (Pm) generation. However, the *in vivo* role of PAI-1 in inactivating uPA and limiting the generation of Pm during cardiac fibrosis remains to be established.

Aims: To test the hypothesis that cardiac fibrosis phenotypes observed in PAI-1^{-/-} mice will be alleviated in the absence of either uPA or active Pm.

Methods: An Angiotensin(Ang)II-Aldosterone(Ald)-induced model of cardiac fibrosis was utilized in wild-type (WT), PAI-1 deficient (PAI-1^{-/-}), uPA deficient (uPA^{-/-}), PAI-1 and uPA deficient (PAI-1^{-/-}/uPA^{-/-}), Pg^{S743A/S743A} (a mouse line that expresses inactive plasmin but normal levels of zymogen plasminogen), and PAI-1^{-/-}/Pg^{S743A/S743A} mice. qRT-PCR and immunohistochemical analysis were used to determine the inflammation and cardiac fibrotic severity in heart tissues.

Results: Four weeks after AngII-Ald infusion, PAI-1^{-/-} mice developed severe cardiac fibrosis. However, a marked reduction in cardiac fibrosis was observed in PAI-1^{-/-}/uPA^{-/-} double knockout mice that was associated with reduced inflammation, lower expression levels of TGF-β and proteases associated with tissue remodeling, and diminished Smad2 signaling. Moreover, similar ablation in cardiac fibrosis was seen in PAI-1^{-/-}/Pg^{S743A/S743A} mice.

Conclusion: The present work provides *in vivo* evidence that the mechanism underlying PAI-1's cardioprotective effects is associated, at least in part, by inhibiting the uPA-mediated activation of Pg. These observations also provide new insights into the mechanisms of cardiac fibrosis and will serve to identify new therapeutic approaches toward regulating this disease.

Disclosure of Interest: None declared.

OR422

Protective role of Protease Nexin-1 against lung fibrosis in bleomycin-treated mice

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Background: Idiopathic pulmonary fibrosis (IPF) is a devastating form of interstitial lung disease associated with an accumulation of extracel-

lular matrix deposition in lungs. Dysregulation of both coagulation and fibrinolysis systems is known to be involved in the development of IPF.

Aims: Since a tissue serpin, named serpinE2 or Protease Nexin-1 (PN-1), exhibits both anticoagulant and antifibrinolytic properties and has recently been shown to be overexpressed in lungs during IPF, we examined the role of PN-1 in the development of IPF.

Methods: We compared bleomycin-induced lung fibrosis on the survival of Wild-Type (WT) and PN-1-deficient mice (KO). Collagen content was analyzed in lung tissue sections by Masson's trichrome and Picro-red Sirius staining, as well as in snap-frozen lung tissue using the Sircol collagen assay. Collagen I and III and fibronectin were quantified by qRT-PCR and immunoblotting. Pro-inflammatory and pro-fibrotic cytokines were measured by ELISA in the bronchoalveolar lavages of the mice.

Results: After bleomycin treatment, PN-1-KO mice displayed a higher mortality rate than WT mice. PN-1 deficiency exacerbates bleomycin-induced lung overexpression of collagen type I and III and fibronectin at both messenger and protein levels. In addition, the aggravation of bleomycin-induced lung fibrosis associated with PN-1-deficiency was accompanied by significant increases in inflammatory cell recruitment and in pro-inflammatory (IL-2 and IL-6) and pro-fibrotic (IL-4) cytokine levels, in their bronchoalveolar lavage fluids. Because PN-1 is the most potent tissue inhibitor of thrombin, the direct thrombin inhibitor argatroban was administered to the mice and led to a significant improvement of the survival of PN-1-KO mice treated with bleomycin.

Conclusion: The presence of PN-1 in lungs plays a protective role indicating that PN-1 regulates the extent of pulmonary fibrosis by inhibiting thrombin pleiotropic cellular functions.

Disclosure of Interest: None declared.

OR423

An anti-staphylocoagulase monoclonal antibody inhibits prothrombin activation and prolongs survival in mice following *Staphylococcus aureus* infection

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Background: Prothrombin and fibrinogen play important roles in pathogenesis of certain bacterial infections. Controlling host coagulation might represent a new strategy for fighting infectious diseases. Staphylocoagulase (SC), a protein secreted by the human pathogen *Staphylococcus aureus*, activates prothrombin by insertion of the N-terminal Ile1-Val2 residues of SC and formation of a salt bridge with Asp194 (chymotrypsinogen numbering) of prothrombin. This induces formation of the substrate binding site and the oxyanion hole required for catalysis and fibrinogen cleavage. This activation mechanism is significantly different than prothrombinase activation of prothrombin which results in proteolytic cleavage of the zymogen.

Aims: In the current study, we tested the hypothesis that blocking SC activation of prothrombin could have therapeutic value for treatment of coagulase positive *S. aureus* infections.

Methods: We produced a murine monoclonal antibody (GMA-2105) directed against a peptide representing SC(1-10).

Results: Both full-length and Fab fragments of GMA-2105, inhibited *S. aureus* plasma clotting, blotted native staphylocoagulase from cultures, and bound with nM affinity to recombinant SC(1-246). In a lethal sepsis murine model, GMA-2105 prolonged survival over control groups (buffer or isotype-matched irrelevant antibody) following injection with coagulase-positive *S. aureus* strain Tager 104. The recombinant chimeric antibody, consisting of murine variable and

human constant regions, bound with indistinguishable affinity to SC (1–246) as the murine antibody measured by direct binding and inhibition of prothrombin activation assays.

Conclusion: Using an antibody to block the ability of *S. aureus* to form fibrin could produce an important chink in the armor of this deadly and widespread pathogen. This approach represents a new way to combat dangerous *S. aureus* infections by eliminating this pathogen's ability to harness prothrombin activation.

Disclosure of Interest: K. Begins Employee of: Green Mountain Antibodies, W. Church Shareholder of: Green Mountain Antibodies, Employee of: Green Mountain Antibodies, A. Maddur: None declared, P. Panizzi: None declared, P. Bock: None declared.

OR424

Protein disulfide isomerase released during thrombus formation cleaves a disulfide bond in plasma vitronectin and enables its binding to alphavbeta3 integrin

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Background: Protein disulfide isomerase (PDI) catalyzes formation and isomerization of protein disulfide bonds. It is secreted by platelets and endothelial cells following vascular injury and is required for thrombus formation. However, the mechanism by which PDI participates in thrombus generation is unknown. We hypothesize that PDI plays a novel role in triggering the activation of critical components required for thrombus formation by modification of disulfide bonds in substrate proteins that leads to functional change.

Aims: Here, we identify PDI substrates in plasma and determine the functional significance of PDI-catalyzed changes in the covalent structure of these substrate proteins.

Methods: Mechanism-based kinetic trapping was used to identify substrate proteins targeted by PDI reductase activity. We used active site mutants of PDI that can form stable mixed disulfide complexes with their substrates then employed mass spectrometry, two dimensional SDS electrophoresis and Western blotting to identify PDI substrates from plasma.

Results: We demonstrate that PDI has numerous reductase substrates including vitronectin. Plasma derived- but not platelet vitronectin is a substrate for PDI. ERp57, a close homolog of PDI, does not reduce plasma vitronectin. Maleimide pegylation and gel shift assay show PDI reduces a single disulfide bond on plasma vitronectin. We demonstrate by solid phase and cellular assays that plasma vitronectin reduced by PDI binds to its receptor, $\alpha_v\beta_3$ integrin, whereas native plasma vitronectin does not.

Conclusion: Extracellular PDI triggers the reduction of an inert form of vitronectin, leading to complex formation with $\alpha_v\beta_3$ and subsequent assembly of additional vitronectin-binding proteins including anti-thrombin III and plasminogen activator inhibitor-1. We propose that PDI-catalyzed reduction is a general mechanism targeting multiple proteins involved in thrombus formation for the rapid regulation of the initiation of hemostasis.

Disclosure of Interest: None declared.

OR425

Identification of alpha-1 proteinase inhibitor variants with enhanced specificity for activated factor XI over thrombin via combined phage display and bacterial lysate screening

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Background: Alpha-1 proteinase inhibitor (API) is the most abundant serpin in plasma. API variant M358R [P1 site of the reactive centre loop (RCL)] inhibits factor XIa (FXIa) and thrombin, unlike wild-type API. The relationship between RCL structure and serpin target protease specificity remains incompletely understood.

Aims: To identify API M358R variants reacting more rapidly with FXI than with thrombin.

Methods: A library of API M358R variants randomized at residues 352–356 (P7-P3) was constructed in T7 bacteriophage, fused to the 3' end of the capsid 10B gene. 10^9 plaque-forming units were screened with 0.5 nM FXIa, and then captured using biotinylated anti-FXIa IgG and streptavidin-coated magnetic beads. *E. coli* BLT 5403 was directly infected with bead-captured phage. After 5 rounds of such biopanning, the RCL inserts of FXIa-selected phage were plaque-purified and sequenced, or transferred en masse to plasmid pBAD-API for soluble bacterial expression. After lysis, API binding to immobilized FXIa or thrombin was quantified via ELISA. Selected purified variants were characterized kinetically.

Results: Of 41 quintuply-selected phage, 13 encoded QSIIS and 7 CLEVE. Of 16 bacterial colonies, 5 encoded CLEVE and 2 QSIIS. Both variants bound FXIa more avidly than thrombin in lysate capture assays. The second order rate constants (k_2) of FXIa inhibition of CLEVE, QSIIS and wild-type (FLEAI) API M358R variants were: $8.57 \pm 1.3 \times 10^4$; $2.01 \pm 0.35 \times 10^5$; and $1.92 \pm 0.14 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ ($n = 11 \pm \text{SD}$). For thrombin, k_2 values were: $3.3 \pm 0.1 \times 10^3$; $1.00 \pm 0.15 \times 10^3$; and $1.60 \pm 0.78 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ ($n = 5-7$). CLEVE and QSIIS variants reacted 22- and 1.7-fold more rapidly with FXIa than thrombin.

Conclusion: Combining phage display of API libraries with coagulation factor-specific capture assays identified API M358R (P7-P3 CLEVE), with markedly enhanced specificity for FXIa. Further increases in specificity of API variants for FXIa over thrombin could be achieved by incorporating negative selection steps.

Disclosure of Interest: None declared.

Blood coagulation tests – II

OR426

Analysis of factor XIa, factor IXa and tissue factor activity in burn patients

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Background: An elevated procoagulant activity has been observed in trauma patients. In part, this increased activity is related to tissue factor (TF) located on blood cells and microparticles. However, quantitated levels of TF in trauma patient plasma indicate that there are other contributor(s) to the procoagulant activity. We hypothesize that factor (F)XIa and FIXa are responsible for an additional activity in burn patients.

Aims: To analyze plasma from burn patients for the presence of FXIa, FIXa and TF and quantitate their concentrations.

Methods: Multiple time-point citrate plasma samples from 56 burn patients (total number of samples analyzed was 463; up to 20 time-

point samples per patient collected in 3 weeks after admission) were evaluated in a thrombin generation assay using anti-FXIa, FIXa and TF monoclonal inhibitory antibodies.

Results: Most patients were male (43/56). The age of patients varied between 18 and 77 years (median 37 years) and 8 patients did not survive their injuries. At the time of admission, 9 patients (16%) had active TF, 54 (96%) had FXIa and 49 (87%) had FIXa in their plasma. Twenty-four patients (43%) without TF activity at the 0 time-point developed that activity in following days. As for FXIa and FIXa, 100% of patients had FXIa and 96% had FIXa at one or more time-points. Overall, TF activity was observed in 97 (21%), FXIa in 417 (90%) and FIXa in 245 (53%) plasma samples. The concentration of TF was relatively low and varied between 0.1 and 2.1 pM, whereas that of FXIa was significantly higher and in some plasma samples exceeded 100 pM. The majority of samples with FIXa activity had that protein at sub-nanomolar concentrations. No TF, FXIa and FIXa activity was detected in plasma from healthy individuals.

Conclusion: The majority of plasma samples from burn patients have detectable FXIa and FIXa activity, with significant fraction of them having active TF as well. The concentration of all three proteins in patient plasma varies in a wide range.

Disclosure of Interest: None declared.

OR427

The whole blood thrombin generation test predicts bleeding risk in patients taking vitamin K antagonists

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Background: Vitamin K antagonists (VKAs) have been the mainstay of oral anticoagulant treatment for the past 60 years. Bleeding is the most prevalent adverse effect of this therapy, with 1–4% of patients subject to major bleeding episodes. Up to this point no laboratory assays are available to predict patients at increased risk for bleeding.

Aims: We investigated whether thrombin generation (TG) tests might detect bleeding risk in patients taking VKAs.

Methods: This prospective study included 129 patients taking VKAs for over 3 months before blood collection and who signed informed consent. TG was determined in whole blood (WB), platelet rich and platelet poor plasma. Hematocrit (Hct) and hemoglobin (Hgb) concentration were determined. The International Normalized Ratio (INR) was defined and fibrinogen levels were measured (Clauss method).

Results: Forty four clinically relevant bleeding episodes were seen in 26 patients (20%), mean follow-up after blood collection: 15.5 months. No differences were found in endogenous thrombin potential (ETP) or peak height in plasma, nor did INR values differ. Interestingly, implementing TG in WB we did find a significantly lower ETP ($P < 0.01$) and peak ($P < 0.05$) in patients with bleeding (median: 183 nM min and 24 nM, respectively) compared to patients without bleeding (median: 256 nM min and 39 nM). The area under the curve (AUC) of the Receiver Operating Curve (ROC) indicated that the ETP (AUC: 0.70) and peak (AUC: 0.64) were significantly ($P < 0.05$) associated with a bleeding tendency. We assessed whether these differences were associated with age, Hct, Hgb or fibrinogen levels, but these parameters did not vary significantly between the two groups.

Conclusion: WB-TG is associated with an increased risk for bleeding in patients on VKA treatment and has a predictive value (based on AUC of ROC). INR and plasma-TG did not discriminate for bleeding risk. There were no significant differences in age, Hct, Hgb or fibrinogen levels that explained the difference in bleeding risk.

Disclosure of Interest: None declared.

OR428

Comparative *in vitro* evaluation of thrombin generation assay (TGA) after spiking fresh frozen plasma (FFP) or prothrombin complex concentrate (PCC) to samples from patients undergoing orthotopic liver transplantation (OLT)

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Background: The haemostatic management of patients during OLT is not well defined. Despite some discrepancies between the bleeding risk and results of standard coagulation assays, fresh frozen plasma (FFP) is often transfused to correct observed biological anomalies. However, this practice remains controversial, and infusion of prothrombin complex concentrate (PCC) has recently been proposed.

Aims: In this context, the aim of this preliminary study was to comparatively evaluate the *in vitro* effects of PCC and FFP on thrombin generation assays (TGA) performed on plasma samples collected during OLT.

Methods: Ten patients, 7H/3F, mean age 62 years, Child-Pugh score min-max 8–13, prothrombin time (PT) min-max 32–62%, were included. Blood samples were taken before incision (P1), at the end of hepatectomy (P2), before portal unclamping (P3) and 30 min after reperfusion (P4). TGA (CAT-method FT 5 pm PLP 4 μ M) were performed on platelet-poor plasmas before and after addition of FFP (5 mL kg⁻¹) or PCC (12.5 UI kg⁻¹ or 25 UI kg⁻¹). Results obtained were compared with those of healthy subjects ($n = 29$; 22H/7F) (Mann and Withney test) and for each sample tested, before and after spiking with FFP or PCC (Wilcoxon test).

Results: TGA parameters at P1 were similar in patients and controls: ETP (min-max) = 939–1584 nM*min vs. 1040–1914 nM*min in controls ($P = 0.24$) and did not vary at P2 and P3. However at P4, ETP significantly decreased: min-max = 678–1222 nM*min ($P < 0.0001$) and was not corrected by the addition of FFP. In contrast, the lowest dose of PCC induced a significant increase of ETP at P4: min-max = 1234–2587 nM*min ($P = 0.002$). Importantly, PCC also induced a marked hypercoagulability when added to plasma collected at P1: ETP (min-max) = 1920–3083 nM*min $P = 0.002$.

Conclusion: The failure of FFP to correct ETP *in vitro* argues for a non-systematic use of this product during OLT. In contrast, the use of PCC could be considered at the moment of reperfusion but not earlier because of a potential thrombotic risk.

Disclosure of Interest: None declared.

OR429

Effects of apixaban on PT and APTT as determined with a full range of reagents: results of a UK NEQAS exercise

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Background: PT and APTT reagents vary in their sensitivity to apixaban. Guidelines recommend that laboratories should be aware of the sensitivity of their coagulation screening tests to the presence of apixaban. Data are needed on the full range of PT/APTT reagents in routine use.

Aims: To assess the effects of a range of apixaban concentrations within and above the expected clinical range on prothrombin time (PT) and activated partial thromboplastin time (APTT) as determined

by all reagents in use by participants in UK NEQAS Blood coagulation.

Methods: The same pooled normal plasma was spiked with 0, 33, 164, 398 and 737 ng mL⁻¹ apixaban (provided by Bristol-Myers Squibb), lyophilised in 1 mL aliquots, and despatched to PT/APTT participants in the UK National external Quality Assessment Scheme (NEQAS) for Blood Coagulation, apixaban concentrations were determined by anti Xa/mass spectrometry. The median ratio of results for each reagent group was calculated (test/mid point normal range). Results were received from > 600 centres (~60% UK, 40% non UK).

Results: Twenty different PT and 18 different APTT reagents were used. The sample containing no apixaban had normal PT and APTT as expected. PT Results: 33 ng mL⁻¹ – all reagent medians normal; 164 ng mL⁻¹ median PT ratios ranged from 1.08 to 1.46; 398 ng mL⁻¹ – range 1.15–2.01; 767 ng mL⁻¹ – range 1.25–2.91. Innovin and Thromborel S were the least sensitive reagents. Recombiplastin 2G was the most sensitive. APTT Results: 33 ng mL⁻¹ – median ratios ranged from 1.04 to 1.28; 164 ng mL⁻¹ – range 1.16–1.43; 398 ng mL⁻¹ – range 1.26–1.76; 767 ng mL⁻¹ – range 1.33–1.90.

Conclusion: Prolongation of PT and APTT by apixaban concentrations of 398 and 767 ng mL⁻¹ varied markedly between reagents. At 164 ng mL⁻¹ PT/APTT were normal with Innovin/Actin FS and showed very minor prolongation with Recombiplastin 2G/Synthasil. Normal PT and APTT could occur at expected peak levels and interpretation of PT and APTT results in the presence of apixaban must take account of the reagents used.

Disclosure of Interest: None declared.

OR430

Prothrombotic mechanisms in a setting of peri-operative bridging with low molecular weight heparins in patients undergoing surgery

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Background: Bridging vitamin K antagonists (VKA) comprises peri-procedural substitution by low molecular weight heparins (LMWH), and is associated with a 3-fold increased bleeding risk.

Aims: To assess the effects of bridging on global and specific coagulation assays in relation to bleeding risk.

Methods: Blood was sampled daily from day -3 to day +5 in 13 patients on chronic VKA who were bridged for a planned intervention. In addition to INR and anti-Xa activity, thrombin generation (TG) was quantified, with further assessment of resistance to activated protein C (APC) as well as single clotting factor levels. The study was ethically approved.

Results: The effect of VKA was absent at the time of intervention (mean INR 1.0 (SD 0.1, range 0.9–1.2); the effect of LMWH was still evident (mean anti-Xa at day 0; 0.19 units mL⁻¹ (SD 0.20 mL, range < 0.05–0.60); 11/13 participants (84.6%) had residual anti-Xa levels of ≥ 0.05 IU mL⁻¹, and 2/13 (15.4%) of > 0.5 IU mL⁻¹. In spite of residual anti-Xa activity, the intervention caused a 2–3 fold increase in TG at day 0, sustaining until day +5. Of all factors, FXI had the strongest correlation with TG ($r \pm 0.6$ for peak and endogenous thrombin potential). Thrombomodulin induced APC resistance (reference 50%) increased from 10.0% (SD 9.2) at day -3 and to 18.2% (SD 9.5) at day 0, $P = 0.02$. After surgery, FVIII and fibrinogen were significantly increased ($P < 0.001$). Correlations between INR and TG were moderate ($r = -0.4$, $P = 0.21$), and strong between

anti-Xa levels and TG ($r = -0.8$, $P = 0.001$). 4/13 patients experienced a major bleed, and no TE.

Conclusion: In spite of residual anti-Xa activity, there was a marked increase in TG related to surgery. 3 prothrombotic mechanisms are exposed: FXI dependent TG, reduced activity of the APC pathway and postoperative rise in FVIII and fibrinogen. For the complex peri-operative hemostasis management the value of TG to monitor the hemostatic balance merits further study.

Disclosure of Interest: None declared.

Fibrinogen and Fibrin II

OR431

Sequencing of *FGA*, *FGB* and *FGG* reveals both novel and previously described mutations in patients with congenital fibrinogen deficiencies

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Background: Fibrinogen plays a critical role in the formation of the final hemostatic clot. Mutations in three genes (*FGA*, *FGB*, *FGG*) located continuously on chromosome 4q23 can lead to qualitative or quantitative fibrinogen deficiencies. Intriguingly, the phenotype of fibrinogen defects is variable from none to severe and includes symptomatic bleeding, clotting or both.

Aims: We sought to correlate genotype with phenotype in fibrinogen defects to understand the effect of these mutations on fibrinogen activity and disease.

Methods: Perform Sanger sequencing of the three fibrinogen genes on genomic DNA from 16 patients. Bleeding events were defined as excessive mucocutaneous, joint or intracranial bleeding. Clotting events were defined as arterial or venous thromboembolism.

Results: In 16 patients, we identified 12 fibrinogen mutations: four novel and eight previously described. Patients were either asymptomatic ($N = 7$), or had a history of bleeding ($N = 4$), thromboembolic events ($N = 2$), or both ($N = 3$). Novel mutations identified were α p.Met1Val, α p.Trp33Stop, β p.Gly272Arg, and γ p.Ala289Asp. The α p.Met1Val mutation disrupted the start codon resulting in undetectable plasma fibrinogen levels and bleeding phenotype. Six patients with mutations in the C-terminus region of *FGG*, which has been proposed to be involved with γ dimerization, had decreased functional fibrinogen levels (mean 77 mg dL⁻¹) and normal fibrinogen antigen levels (mean 192 mg dL⁻¹), indicating a qualitative defect [Mosesson MW, 1995]. Interestingly, these mutations (four Bergamo II mutations [γ p.Arg275His] and a novel mutation [two γ p.Ala289Asp]) resulted in variable phenotypes (asymptomatic, bleeding, clotting, or both).

Conclusion: Fibrinogen disorders are difficult to characterize due to their phenotypic variability. Genetic characterization is essential to understand the genotype/phenotype correlation. This study will contribute to the characterization of fibrinogen mutations and relationship to fibrinogen laboratory and clinical phenotypes.

Disclosure of Interest: None declared.

OR432

Hereditary afibrinogenemia – long-term observation of a highly thrombogenic condition and its managementNagler M^{1,2,3}, Kremer Hovinga JA^{1,2}, Alberio L^{1,2,4}, Peter-Salonen K^{1,2,5}, von Tengg-Kobligk H⁶, Lottaz D² and Lämmle B^{1,2,7}¹University Clinic of Haematology and Central Haematology Laboratory, Inselspital Berne University Hospital; ²Department of Clinical Research, University of Berne, Berne, Switzerland;³Laboratory of Clinical Thrombosis and Haemostasis, and Cardiovascular Research Institute, Maastricht University Medical Center, Maastricht, The Netherlands; ⁴Haematology Service and Central Laboratory, University Hospital of Lausanne CHUV, Lausanne; ⁵Swiss Medical Assessment- and Business-Center (SMAB) AG; ⁶Institute for Diagnostic, Interventional and Pediatric Radiology, Inselspital University Hospital, Berne, Switzerland; ⁷Center for Thrombosis and Haemostasis, University Medical Center, Mainz, Germany**Background:** The high risk of arterial and venous thromboembolism in patients with hereditary afibrinogenemia (HA) is neither understood, nor is a safe and effective treatment established.**Aims:** To report on long-term data of five HA patients with severe thromboembolic complications, possibly due to lacking anti-thrombin activity of fibrinogen (fbg)/fibrin.**Methods:** Five patients were followed for 10–54 years.**Results:** HA was diagnosed in two brothers, their two cousins and one unrelated female patient by plasma fbg determination. Levels of D-dimers were below $45 \mu\text{g L}^{-1}$. A homozygous 11 kb deletion in the *FGA* gene was detected in all patients, who had recurrent bleeding complications and were treated with fbg concentrates every 4–6 weeks. Four patients developed recurrent and extensive arterial and venous thromboembolism, beginning at ages of 25–38 years. In case of acute thrombotic events, thrombin-antithrombin levels (TAT) were elevated above $60 \mu\text{g L}^{-1}$. In two patients, treatment was initiated to achieve constant fbg trough levels of $\geq 0.5 \text{ g L}^{-1}$. It consisted of regular fbg replacement in one patient (1–2 g two or three times a week) and orthotopic liver transplantation performed in another patient. Fbg trough levels rose to $\geq 0.5 \text{ g L}^{-1}$. Two to four hours after fbg replacement, D-dimer levels increased to $100\text{--}200 \mu\text{g L}^{-1}$, TAT decreased to values between 5 and $15 \mu\text{g L}^{-1}$. Radiological imaging documented resolved arterial thrombi after 6–12 months. No new thromboembolic events were observed for 7–9 years since initiating regular fbg replacement or liver transplantation, respectively.**Conclusion:** Regular fbg replacement therapy or orthotopic liver transplantation seemed to effectively scavenge thrombin, generated during acute phase reactions *in vivo* and reduced the high risk of thromboembolic complications. Our data suggest regular fbg replacement therapy resulting in permanently measurable fbg levels to be a safe and effective treatment option in patients with HA and thromboembolic complications.**Disclosure of Interest:** M. Nagler Grant/Research Support from: CSL Behring, J. Kremer Hovinga Grant/Research Support from: Baxter Bioscience, L. Alberio: None declared, K. Peter-Salonen: None declared, H. von Tengg-Kobligk: None declared, D. Lottaz: None declared, B. Lämmle Grant/Research Support from: Baxter Bioscience.

OR433

Elevated fibrinogen but not fibrinogen gamma prime in abdominal aortic aneurysmal diseaseMacrae F¹, Thomas T¹, Johnson A^{1,2}, Bridge K^{1,2}, Philippou H¹, Scott DJ^{1,2} and Ariens R¹¹Division of Cardiovascular and Diabetes Research LIGHT Labs Leeds, University of Leeds; ²Leeds Vascular Institute, Leeds General Infirmary, Leeds, UK**Background:** Abdominal aortic aneurysm (AAA) represents a chronic inflammatory disease with lethal implications. Elevated plasma fibrinogen concentrations associate with elevated risk of thrombosis, and in a small study of 110 patients were found to be higher in AAA. A fraction of fibrinogen called the γ' chain differs from the γA chain due to a unique 20-amino acid extension at its C-terminus. The γ' chain has been shown to influence clot structure, causing thinner fibres, increased branching and smaller pores.**Aims:** Investigate levels of fibrinogen and fibrinogen γ' in AAA patients and controls.**Methods:** Six hundred and nine AAA patients and 559 age and sex matched controls were recruited in the Leeds Aneurysm Development Study (LEADS). Plasma fibrinogen levels were measured using the Fibrinogen automate assay (based on the Clauss method) and fibrinogen γ' was measured by ELISA using an antibody specific to human fibrinogen γ' (2.G2.H9, Santa Cruz). Other clinical data was recorded for each patient. Results are expressed as median (IQR).**Results:** Fibrinogen levels were higher in patients than controls (3.68 g L^{-1} (3.18–4.31) vs. 3.50 g L^{-1} (3.09–4.08), $P = 0.001$). There was no difference in γ' levels between patients and controls ($334.53 \mu\text{g mL}^{-1}$ (212.16–508.02) vs. $331.00 \mu\text{g mL}^{-1}$ (211.28–510.54) $P = 0.747$). There was a lower percentage of γ' in patients compared with controls but this did not reach significance (9.26% (5.64–13.74) vs. 9.44% (6.04–14.89), $P = 0.173$). γ' levels were positively correlated with CO levels ($r = 0.149$, $P = 0.002$), homocysteine levels ($r = 0.235$, $P = 0.024$) and negatively correlated with HbA1c ($r = -0.124$, $P = 0.004$).**Conclusion:** We found significantly raised levels of fibrinogen in AAA patients in this large case-control study; these likely reflect the underlying inflammatory process associated with AAA. Furthermore, elevated fibrinogen levels in AAA patients may contribute to the increased cardiovascular risk observed in this group of patients. There was no association between AAA and fibrinogen γ' .**Disclosure of Interest:** None declared.

OR434

Role of fibrin(ogen) in hepatocyte proliferation after acetaminophen overdoseKopeck A¹, Joshi N², Towery K¹, Cline-Fedewa H¹, Flick M³ and Luyendyk J¹¹Pathobiology & Diagnostic Investigation; ²Pharmacology & Toxicology, Michigan State University, East Lansing, MI; ³Cancer and Blood Diseases Institute, Division of Experimental Hematology & Cancer Biology, Cincinnati Children's Hospital, Cincinnati, OH, USA**Background:** Acetaminophen (APAP) overdose is the leading cause of drug-induced acute liver failure in the United States. APAP-induced hepatotoxicity in humans and mice is associated with activation of blood coagulation cascade and deposition of polymerized fibrin clots in the liver.**Aims:** Here, we sought to determine whether fibrin(ogen) contributed to hepatocyte proliferation and liver repair after APAP overdose in mice.

Methods: Mice with genetically-imposed complete fibrin(ogen) deficiency (Fib^{-/-}) and heterozygous control mice (Fib^{+/-}) were given a hepatotoxic dose of APAP (300 mg kg⁻¹, ip) followed by analysis of liver injury and hepatocyte proliferation at multiple time points.

Results: Hepatocyte proliferation was markedly reduced in APAP-challenged Fib^{-/-} mice compared to Fib^{+/-} control mice, which paralleled elevated liver necrosis and hepatocellular damage (i.e., serum alanine aminotransferase activity) at 48 and 72 h after APAP overdose suggesting a defect in liver repair. Next, we tested the hypothesis that fibrin(ogen) promotes hepatocyte proliferation after APAP overdose through the leukocyte integrin Mac-1/ $\alpha_M\beta_2$ binding motif, as emerging studies suggest macrophages are essential for liver repair. Here, we utilized Fib $\gamma^{390-396A}$ mice, which express a mutant form of fibrin(ogen) incapable of binding $\alpha_M\beta_2$ integrin. Remarkably, molecular markers of hepatocyte proliferation were nearly absent in APAP-treated Fib $\gamma^{390-396A}$ mice compared to APAP-treated wild-type mice, as early as 24 h after APAP administration.

Conclusion: Overall, the results suggest that hepatic fibrin deposition and engagement of the leukocyte $\alpha_M\beta_2$ integrin by liver macrophages is an important molecular trigger of hepatocyte proliferation and liver repair after experimental APAP overdose.

Disclosure of Interest: None declared.

OR435

Prothrombotic properties of fibrin clots in patients with non-alcoholic fatty liver disease

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Background: Non-alcoholic fatty liver disease (NAFLD) is the most frequent chronic liver disorder in Western countries, and is associated with an increased risk of cardiovascular disease. The mechanisms underlying this association are incompletely understood, but may involve hypercoagulable changes. Alterations in fibrin clot structure have recently been recognized as possible contributors to thrombotic disease.

Aims: To study the fibrin clot structure in patients with NAFLD.

Methods: Blood was drawn from 68 patients with NAFLD (simple steatosis $n = 24$, NASH $n = 22$, and NASH cirrhosis $n = 22$), 20 lean controls, 20 overweight controls (BMI > 25 kg m⁻²), and 15 patients with alcoholic (ASH) cirrhosis. Clots were generated by mixing citrated plasma with thrombin in presence of calcium. Fibrin pore structure was evaluated by permeation analyses, in which the flow rate of a buffer through a fibrin clot is measured. Fibrin density was assessed by calculating the number of fibers/100 μ m using confocal microscopy.

Results: Clot permeability was decreased in overweight controls and patients with NAFLD, indicating more dense clots compared to lean controls. Clot permeability was also decreased in patients with ASH-related cirrhosis, although the decrease in permeability in NASH-related cirrhosis was more extensive. The decreased clot permeability was only to a limited extent explained by increased fibrinogen plasma levels, suggesting structural changes in the fibrinogen molecule. Confocal microscopy revealed a stepwise increase in fiber density from lean controls to overweight controls and patients with simple steatosis to patients with NASH and NASH cirrhosis. Fiber density was also increased in patients with ASH-related cirrhosis compared to lean controls.

Conclusion: Our results show decreased fibrin clot permeability and increased clot density in patients with NAFLD, which may contribute to their increased risk of cardiovascular disease.

Disclosure of Interest: None declared.

Cancer and thrombosis – basic – II

OR436

Anti-tumorigenic effects are exerted by exosomes derived from heparin treated human breast cancer cells and persist even after heparin treatment has been discontinued

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Background: Numerous clinical studies have established that heparin treatment prolongs the survival of cancer patients. In these studies it was also noted that the beneficial effects persisted long after heparin treatment had been discontinued. To date, no evidence has been provided to explain this prolongation of the effect of heparin. Exosomes, stable vesicular structures released by cells are concentrated carriers of genetic and proteomic information thought to play important roles in intercellular communication. We hypothesised that exosomes released from the heparin treated cells could influence cell function even after heparin had been withdrawn.

Aims: To investigate the role of exosomes in contributing to the anti-tumorigenic activity of heparin.

Methods: Human breast cancer cells [MCF-7, MDA-MB231 and cancer enriched stem cell (CSCs)] were used. Exosomes were isolated by ultracentrifugation from culture medium of heparin treated (2 days) cells and also 2 days later after discontinuation of heparin treatment. Exosomes from untreated cells were used as controls. The properties of exosomes were assayed according to the expression profile of miRNAs and pro-tumorigenic protein. The exosomes from heparin treated cells were also used to treat different mammary epithelial cells lines (MECs).

Results: Heparin exerted an anti-tumorigenic effect on exosomes by modulating a number of miRNAs which is relevant to tumorigenesis and cell cycle regulation proteins as well as by reduced expression of pro-tumorigenic proteins in a similar way to our previous reports on breast cancer cells. Exosomes alone exerted an anti-tumorigenic effect when added to MECs which persisted after heparin removal, observed as reduced cell proliferation and modulated the cell cycle profile as well as 12 cell cycle regulatory proteins.

Conclusion: The anti-tumorigenic of heparin is mediated by exosomes and able to persist even after heparin treatment has been discontinued.

Disclosure of Interest: None declared.

OR437

Contribution of anti-GRP78 autoantibodies to prostate cancer progression through tissue factor procoagulant activity

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Background: Prostate cancer (PC) is the most frequently diagnosed cancer in men. PC can increase the risk of thrombosis due to the enhanced expression and/or procoagulant activity (PCA) of tissue factor (TF). We now have evidence that TF expression/PCA can be modulated in PC cancer cells by GRP78, an endoplasmic reticulum (ER)-resident molecular chaperone. Unlike normal cells, we and others have made the surprising discovery that GRP78 is expressed on the surface of many human cancers, including PC, where it functions as a unique signaling receptor to promote cell proliferation. Furthermore, exposure of GRP78 on the surface of PC cells stimulates the production of anti-GRP78 autoantibodies (aGRP autoAB) in PC patients and correlate with accelerated cancer progression, enhanced metastatic potential and reduced survival.

Aims: To investigate the contribution of aGRP autoAB to accelerated PC progression. To investigate aGRP autoAB as a viable target for the treatment of advanced PC.

Methods: Wild type/TF knock-down DU145 PC cells were used. Protein expression was determined using western blotting/qRT-PCR. A NOD/SCID mice were used to investigate the tumour growth model. TF PCA was determined using the continuous assay. PC patient samples were obtained from the Ontario Tumour Bank and St. Joseph's Hamilton.

Results: Pre-prostatectomy patients demonstrated high levels of aGRP autoAB, compared to patients 24 weeks post-op. aGRP autoAB upregulate TF PCA through a mechanism involving depletion of ER Ca²⁺ stores and activate the unfolded protein response (UPR). Further, these autoantibodies were shown to accelerate tumour growth in the NOD/SCID mouse model. Finally, we demonstrate that low molecular weight heparins (LMWH) can disrupt the aGRP autoAB binding to cancer cells and reduce TF PCA.

Conclusion: High levels of aGRP autoAB correlate with cancer stage, increase TF PCA *in vitro*, and promote cancer growth in the NOD/SCID mouse model system. LMWH impairs this interaction and significantly reduces TF PCA on the PC cells.

Disclosure of Interest: None declared.

OR438

Cancer cells BXP3 and MCF7 differentially reverse the inhibition of thrombin generation by apixaban, fondaparinux and enoxaparin

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Background: Cancer cells may alter the efficiency of the antithrombotic agents.

Aims: To explore this possibility, we studied the influence of pancreas adenocarcinoma cells (BXP3) and human breast carcinoma cells (MCF7) on the antithrombotic efficiency of apixaban, fondaparinux and enoxaparin.

Methods: Samples of platelet poor (PPP) or platelet rich plasma (PRP) spiked with apixaban, fondaparinux or enoxaparin were added in micro wells carrying cancer cells and assessed for thrombin generation. In the control experiment thrombin generation was triggered with tissue factor reagent.

Results: The three antithrombotics inhibited thrombin generation in a concentration dependent manner. The BXP3 and MCF7 cells reversed in a different intensity the effect of the studied agents. According to the histological type of the cancer the antithrombotic efficiency of apixaban was preserved or partially reversed. Fondaparinux, was more vulnerable to the presence of cancer cells as compared to apixaban. The effect of BXP3 or MCF7 cells on the antithrombotic potency of enoxaparin was of similar magnitude as that on apixaban.

Conclusion: The type of cancer cells is determinant for the antithrombotic efficiency of the specific factor Xa inhibitors. In contrast it does not significantly influence the potency of enoxaparin. The present study shows that the impact of the type of cancer cells on the antithrombotic activity of the specific Xa inhibitors should not be neglected. This has to be taken into consideration for the design of dose-finding studies of the direct orally active FXa inhibitors in patients with different histological types of cancer.

Disclosure of Interest: None declared.

OR439

Inhibition of microparticle induced tumor cell migration by LMWH tinzaparin

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Background: Recent meta-analyses have shown that the survival benefit in cancer treatment studies is marginally in favor of an additional LMWH treatment. However, some excellent clinical studies found a clear and highly significant survival benefit. These divergent results indicate a functional difference of tumor cell biology between the patients. Retrospective analyses indicate that additional LMWH treatment improves outcome in non-metastatic stages, cancers with lower proliferation and cancers responsive to chemotherapy.

The active formation of tissue factor-bearing microparticles (TFMPs) is one cause of the systemic activation of the coagulation system in cancer patients. TFMPs in turn can affect the biological behavior of tumor cells by activating the PAR2/MAP kinase pathway via the TF/FVIIa/FXa complex.

Aims: We present evidence that tinzaparin, a LMWH with particularly high TFPI-releasing potency, is able to interrupt this fatal chain of action.

Methods: We used malignant effusions from three cancer patients (colorectal, breast, NSCL cancer) as a source for TFMPs and the migration of human pancreas carcinoma cells as a surrogate parameter for their metastatic potency.

Results: (1) Centrifuged cell-free effusions significantly promote tumor cell migration, (2) tinzaparin significantly inhibits this effect, (3) effusions contain high amounts of TFMPs, (4) TF activity of TFMPs is inhibited by TFPI, (5) tinzaparin induces TFPI release from tumor cells, (6) rTFPI inhibits effusion-induced tumor cell migration, (7) PAR2 inhibitor and ERK inhibitor suppress effusion-induced tumor cell migration.

Conclusion: We present a possible molecular explanation for the survival benefit observed in some clinical cancer studies with LMWHs. We used cellfree centrifuged malignant effusions as a source for microparticles, which excludes e.g. endothelial P-selectin as a target of heparin action. TFMPs might be a surrogate parameter for clinical studies investigating a possible survival benefit of LMWHs.

Disclosure of Interest: None declared.

OR440

Novel regulation of TFPI expression by oestrogens in breast cancer cells through micrornas

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Background: Tissue factor pathway inhibitor (TFPI) has been linked with breast cancer pathogenesis. We have recently reported TFPI mRNA levels to be downregulated by oestrogens in a breast cancer cell line (MCF7) through the oestrogen receptor α (ER α). Accumulating evidence indicates that activation of ER α signalling by oestrogens may modulate the expression of target genes indirectly through microRNAs (miRNAs).

Aims: To examine if miRNAs are involved in the oestrogenic regulation of TFPI.

Methods: Computational analysis of *TFPI* (NM_006287) 3' untranslated region (UTR) identified conserved binding sites for miR-27a/b and miR-494. Transient miR-27a/b and miR-494 overexpression or inhibition was achieved by mimic or inhibitor transfection, respectively. Direct targeting of *TFPI* 3' UTR by miR-27a/b and miR-494 was determined by a luciferase reporter assay in human colon cancer cell line deficient for Dicer (DKO). Effect of 1–100 nM 17 α -ethinylestradiol (EE2) and 1 μ M fulvestrant (ICI 182,780), an ER α antagonist, on relative miR-27a/b and miR-494 levels in breast cancer cells (MCF7) were determined by qRT-PCR.

Results: Oestrogens lead to a significant upregulation of relative miR-27a/b and miR-494 levels in MCF7 cells following treatment with EE2 and this was successfully counteracted by fulvestrant. *TFPI* mRNA levels were significantly downregulated or upregulated by miR-27a/b and miR-494 mimics and inhibitors, respectively. Preliminary data indicate impaired oestrogen mediated downregulation of TFPI in anti-miR-27a/b and anti-miR-494 transfected cells prior to addition of oestrogens. Luciferase experiments showed direct targeting of miR-27a/b on *TFPI* mRNA in DKO cells.

Conclusion: Our results strongly suggest a role of miR-27a/b and miR-494 in modulating TFPI expression and their possible involvement in oestrogenic regulation of TFPI. Studies on the clinical relevance of miR-27a/b and miR-494 involvement in the oestrogenic regulation of TFPI are in progress.

Disclosure of Interest: None declared.

Fibrinolytic system – II

OR441

Thrombin-activatable fibrinolysis inhibitor (TAFI) peptides selectively modulate TAFI activation by thrombin or the thrombin-thrombomodulin complex

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Background: Thrombin-activatable fibrinolysis inhibitor (TAFI) is a risk factor for coronary heart disease. TAFI is proteolytically activated by thrombin, the thrombin-thrombomodulin (T-TM) complex or plasmin. In normal hemostasis, the activated form protects a blood clot from premature lysis. Specifically targeting TAFI activation can therefore be a powerful strategy for the treatment of both thrombosis and bleeding.

Aims: The aim of this study was to generate TAFI peptides that specifically modulate TAFI activation.

Methods: Thirty-four overlapping peptides, based on the amino acid sequence of TAFI, were synthesized. The enzyme kinetics were determined to quantify the effects of these peptides on TAFI activation by thrombin or the T-TM complex.

Results: Four peptides (peptides 2, 18, 19 and 34) inhibited TAFI activation. Peptides 18 (Gly205-Ser221) and 19 (Arg214-Asp232) inhibited TAFI activation by thrombin and the T-TM complex. Furthermore, these peptides bound to thrombin, suggesting that Gly205-Asp232 of TAFI is involved in the interaction with thrombin. Peptides 2 (Arg12-Glu28) and 34 (Cys383-Val401) inhibited TAFI activation by the T-TM complex with IC₅₀ values of 41.9 $\pm$ 9.1 and 6.1 $\pm$ 0.9 μ M, respectively. However, no inhibition was observed when TAFI was activated by thrombin in the absence of TM. This suggests that amino acids Arg12-Glu28 and Cys383-Val401 in TAFI interact with the T-TM complex. Furthermore, substitution of Trp395 to alanine in peptide 34 led to an almost complete loss of its inhibitory effect, suggesting an important role for Trp395 in TAFI activation by the T-TM complex.

Conclusion: We generated TAFI-derived peptides that can selectively modulate TAFI activation by thrombin or T-TM and that can be used for the design of more potent, specific inhibitors. Furthermore, since the TM concentration varies throughout the vasculature (i.e. higher in smaller vessels), such specific inhibitors provide an opportunity to locally regulate TAFI activation.

Disclosure of Interest: None declared.

OR442

Lysine residues 42, 43, and 44 of thrombin activable fibrinolysis inhibitor contribute to its activation by the thrombin-thrombomodulin complex in a cooperative fashion

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Background: The thrombin-thrombomodulin (TM) complex activates thrombin activable fibrinolysis inhibitor (TAFI) more efficiently than thrombin alone. Previously, the TM dependence of TAFI activation was shown to involve three consecutive Lys residues (K42, K43 and K44) in its activation peptide domain.

Aims: To determine the individual contribution of K42, K43 and K44 of TAFI to its activation by thrombin-TM.

Methods: Wild-type TAFI and variants with single, double or triple Lys to Ala mutations in all possible combinations were generated and their activation by thrombin-TM or thrombin alone were compared as were their activities in a clot lysis assay performed in TAFI-depleted plasma.

Results: Replacement of one, two, or three Lys residues with Ala decreased the catalytic efficiency of TAFI activation by thrombin-TM by 58%, 68%, and 79%, respectively, with the increase in Km exerting the greatest overall effect. When activated by thrombin alone, the TAFI variants had similar activation kinetics. These findings suggest that the three Lys residues of TAFI contribute to its interaction with thrombin-TM in a progressive and TM-dependent manner. In the clot lysis assay, replacement of one, two, or three Lys residues with Ala increased the TAFI concentration required to reach half-maximal prolongation of lysis times by 3-, 4- and 15-fold, respectively; findings consistent with a threshold-like effect on TAFI-mediated attenuation of clot lysis.

Conclusion: The triple Lys cluster within the TAFI activation peptide serves as an exosite for binding to thrombin-TM. The functional consequence of replacement of one, two or three Lys residues with Ala demonstrates threshold-like behaviour, suggesting that each Lys residue contributes to efficient TAFI activation and attenuation of clot lysis.

Disclosure of Interest: None declared.

OR443

Elucidation of the molecular mechanisms of two nanobodies that inhibit TAFI activation and TAFIA activity

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Background: Thrombin activatable fibrinolysis inhibitor (TAFI) is a procarboxypeptidase. TAFI is activated to TAFIa, either by thrombin (T), plasmin or most efficiently by the thrombin-thrombomodulin complex (T/TM). TAFIa exerts antifibrinolytic properties. Our laboratory has previously generated and characterized nanobodies (Nbs) that inhibit TAFI activation and/or TAFIa activity through different mechanisms.

Aims: to elucidate the molecular mechanisms of inhibition using X-ray crystallography.

Methods: Recombinant TAFI was expressed in a glycoengineered *Pichia pastoris* strain. Two Nbs with distinct inhibitory properties were selected for structural studies. Nb-a204 interferes with all three modes of TAFI activation, whereas Nb-i83 interferes only with T/TM-mediated activation but also inhibits TAFIa activity. Diffraction data of crystals of the two TAFI/Nb complexes were obtained and the structures were solved by molecular replacement.

Results: The structure of TAFI/Nb-a204 reveals that a204 binds exclusively to the catalytic moiety (CM) of TAFI. The interface is small (658 Å²) and is stabilized by Trp¹⁰² in the CDR3 loop of a204 interacting with a pocket on TAFI formed by helix 6 (Ala⁹⁵-Glu⁹⁹) and helix 8 (Trp¹⁶³-Phe¹⁷⁹). The Nb binding region is in close proximity to the cleavage site (R⁹²-A⁹³). This provides a molecular basis for the mechanism of action of a204, i.e. by blocking accessibility to the cleavage site. The structure of TAFI/Nb-i83 reveals that i83 binds to both the activation peptide (AP) and the CM. i83 interacts with residues close to the presumptive TM binding region in the AP, providing a molecular basis for T/TM-mediated activation interference. The structure also suggests that the ability of i83 to inhibit TAFIa may be a result of i83 bridging the AP to the CM after TAFI activation.

Conclusion: Nanobody a204 and i83 merit further evaluation as potential profibrinolytic drugs. Analysis of these crystal structures may also lead to opportunities for the rational design of small TAFI inhibitors.

Disclosure of Interest: None declared.

OR444

The role of thrombin-activatable fibrinolysis inhibitor in human abdominal aortic aneurysms

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Background: Intra-luminal thrombus is a key factor in Abdominal Aortic Aneurysms (AAA) growth. We previously showed that patients with AAA form dense clots which are resistant to fibrinolysis.

Aims: The aim of this study is to characterise the role of TAFI in human AAA.

Methods: AAA Patients ($n = 250$) and controls ($n = 210$) were recruited in the Leeds Aneurysm Development Study (LEADS). Plasma levels of intact TAFI, TAFI activation peptide (TAFI-AP) and activated/inactivated TAFI (TAFIa/ai) were measured by ELISAs. Clot lysis was measured in the presence and absence of potato-

carboxypeptidase inhibitor (PCI) to determine TAFI activity. TAFI Thr325Ile genotype was determined using rtPCR.

Results: TAFIa/ai and TAFI-AP levels were higher in patients than controls (mean $\pm$ SEM: 27.1 ± 1.4 vs. $18.2 \pm 1.0 \mu\text{g mL}^{-1}$, $P < 0.001$ and 372.3 ± 16.2 vs. $280.0 \pm 11.7 \text{ ng mL}^{-1}$, $P < 0.001$). TAFIa/ai levels were positively correlated with TAFI-AP ($r = 0.3$, $P < 0.001$). The change in lysis time with PCI was lower in patients than controls (44.6 ± 0.9 vs. $51.6 \pm 1.1 \text{ min}$, $P < 0.001$). Intact TAFI levels were not different between patients and controls (13.5 ± 0.2 vs. $13.3 \pm 0.3 \mu\text{g mL}^{-1}$, $P = 0.7$). There was no difference in Thr325Ile genotype distribution between patients and controls, but possession of the Ile allele was associated with increased TAFI activity (50.0 ± 1.1 vs. $45.7 \pm 1.0 \text{ min}$, $P = 0.004$). Blocking TAFI with PCI reduced clot lysis times in patients to rates seen in controls (AAA: 34.3 ± 3.5 reduced to 28.1 ± 1.3 vs. Control: $27.8 \pm 1.1 \text{ min}$). Lysis across all subjects was positively correlated with levels of TAFI-AP ($r = 0.5$ $P < 0.0001$) and intact TAFI ($r = 0.3$ $P < 0.05$).

Conclusion: The increase in TAFIa/ai and TAFI-AP, and decrease in TAFI activity suggest increased TAFI turnover in patients with AAA. The delay in lysis in patients with AAA can be attributed to TAFI. Whether these changes reflect cause or effect remains to be established. Prospective studies are required to fully elucidate the role of TAFI and fibrinolysis in AAA pathogenesis.

Disclosure of Interest: None declared.

OR445

Mechanistic studies and fibrin specificity of streptokinase from *Streptococcus pyogenes*

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Background: Therapeutic streptokinase (SK) is derived from a group C strain of *Streptococci* and is not fibrin specific. However, SK molecules from group A *Streptococci* (GAS) are known to have different mechanisms that involve bacterial cell surface receptors such as M1 and PAM, which bind fibrinogen (Fgn) and plasminogen (Pgn), respectively.

Aims: To study the mechanism of SK from *S. pyogenes* (M1-type GAS) and understand regulation of plasminogen activation by fibrinogen and fibrin.

Methods: Recombinant therapeutic SK (H46a-SK) and GAS-SK proteins were expressed in *E. coli* and recombinant tPA was expressed in insect cells. Plasminogen activation assays were performed in solution, with and without various fibrinolysis stimulators. Fibrinolysis assays were performed in fibrin clots with ranges of tranexamic acid (TA).

Results: H46a-SK was active in solution against free Pgn, and very poorly stimulated by Fgn or soluble fibrin analogues, including oxidised Fgn, CNBr Fgn fragments or fibrin degradation products (FDP). GAS-SK required stimulation for activity, following a similar pattern to tPA. More detailed analysis over a range of Pgn and Fgn concentrations showed maximum stimulation of 2.5-, 12- and 30-fold for H46a-SK, tPA and GAS-SK, respectively. A 'trigger and bullet' model was developed to explain the stimulation mechanism of GAS-SK by Fgn. In fibrin, the 3 activators had similar K_m values for bound Pgn, 82–113 μM but the V_{max} for GAS-SK was 15- and 24-fold higher than H46a-SK and tPA, respectively. GAS-SK activity was strongly inhibited by TA in the presence of Fgn ($EC_{50} = 14.5 \mu\text{M}$) but less sensitive in fibrin ($EC_{50} = 133 \mu\text{M}$).

Conclusion: GAS-SK may be termed a fibrin-specific Pgn activator like tPA because it demonstrates dramatic stimulation in the presence of fibrin. However, GAS-SK is also strongly stimulated by Fgn, more so than tPA. The response to TA suggests different interactions with Fgn and fibrin for kringle domains of Pgn in complex with GAS-SK.

Disclosure of Interest: None declared.

POSTERS

ADAMTS13 I

PO001-MON

Protective effect of ADAMTS13 haplotype on coronary artery diseaseLasom S^{1,2}, Komanasin N^{2,3}, Settatsian N^{2,3}, Settatsian C^{2,4}, Kukongviriyapan U^{2,4}, Senthong V^{2,4} and Intharapetch P^{2,5}¹Ph.D. Candidate in Biomedical Sciences Program, Graduate School; ²Cardiovascular Research Group; ³Faculty of Associated Medical Sciences; ⁴Faculty of Medicine; ⁵Queen Sirikit Heart Center of the Northeast, Khon Kaen University, Khon Kaen, Thailand**Background:** A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) plays vital roles in preventing excessive spontaneous leukocyte adhesion and reducing vascular inflammation as well as atherosclerotic plaque formation. In addition, ADAMTS13 polymorphisms are related to reduce ADAMTS13 activity. Thus, they may in turn result in atherosclerosis progression.**Aims:** This study aimed to assess the association of polymorphisms and haplotypes of ADAMTS13 gene with coronary artery disease (CAD) risk in Thais.**Methods:** Seven single nucleotide polymorphisms (SNPs) of ADAMTS13 gene including Q448E, rs2073932, P618A, A900V, S903L, rs652600 and rs4962153 were detected in 500 subjects. The subjects were divided into 3 groups; 197 CAD, 136 non-CAD patients and 167 healthy controls. The SNPs were determined by polymerase chain reaction-restriction fragment length polymorphism technique. Haplotype analysis was performed by Arlequin version 3.5 and CAD risk was assessed by using logistic regression analysis.**Results:** The A900V polymorphism was associated with decreased CAD risk compared to healthy controls (OR 0.1; 95% CI 0.0, 0.8). Haplotype analysis indicated that the CAGA (H4) haplotype of Q448E, rs2073932, rs652600 and rs4962153 polymorphisms was less common in CAD compared to non-CAD and healthy controls. Moreover, frequencies of the CAAG (H5) and GGGG (H6) haplotypes were also less frequent in CAD than those in the healthy controls. Interestingly, the H4 haplotype was associated with reduced CAD risk when the reference was either non-CAD (OR 0.2; 95% CI 0.1, 0.5) or healthy controls (OR 0.2; 95% CI 0.1, 0.7). However, the associations of the H5 and H6 haplotypes with CAD risk were not demonstrated.**Conclusion:** This study indicated that the H4 haplotype had a protective effect against CAD risk. This finding suggests that investigating the combination of ADAMTS13 SNPs is more effective than determining a single SNP for prediction of CAD risk.**Disclosure of Interest:** None declared.

PO002-MON

An unbalance between von Willebrand factor and ADAMTS13 following a major partial hepatectomyGroeneveld D¹, Alkozai E¹, Adelmeijer J¹, Porte R² and Lisman T^{1,2}¹Surgical Research Laboratory, Department of Surgery; ²Section of Hepatobiliary Surgery and Liver Transplantation, Department of Surgery, University Medical Center Groningen, Groningen, The Netherlands**Background:** Conventional coagulation tests are frequently prolonged after liver surgery, suggesting a postoperative bleeding tendency. How-

ever, thrombotic complications following a major partial hepatectomy (PH) are not uncommon and can have devastating consequences. Little information is available regarding changes in the platelet adhesive protein von Willebrand factor (VWF) and its cleaving protease a disintegrin and metalloprotease with a thrombospondin type 1 motif (ADAMTS13) following a PH.

Aims: We assessed the status of the primary hemostatic system, specifically the balance between VWF and ADAMTS13 in patients undergoing PH.**Methods:** We collected plasma samples from 17 patients who underwent a major PH and 24 healthy individuals. As a control, we collected samples from 10 patients who underwent a pylorus-preserving pancreaticoduodenectomy (PPPD), which is a procedure of a similar extent, but without a decrease in postoperative synthetic capacity of the liver. We measured plasma levels of VWF and ADAMTS13, VWF activity, and VWF-dependent platelet adhesion.**Results:** VWF levels increased following PH and PPPD and remained high, even 4 weeks following PH. This increase in VWF levels was accompanied by a decrease in ADAMTS13 activity, resulting in a substantially increased VWF/ADAMTS13 ratio which peaked at 7 days following PH. VWF functional activity increased following both procedures, as did VWF-dependent platelet adhesion and aggregate size under conditions of flow. Although similar changes in the VWF-ADAMTS13 axis were also present after a PPPD, changes in the VWF-ADAMTS13 axis were more extensive and more sustained following PH compared to changes following PPPD.**Conclusion:** We observed a hyperactive primary hemostatic system in patients following PH as evidenced by highly elevated plasma levels of functional VWF, combined with decreased ADAMTS13 activity. These alterations in the balance between VWF and ADAMTS13 could contribute to postoperative thrombotic complications, in particular following PH.**Disclosure of Interest:** D. Groeneveld: None Declared, E. Alkozai Grant/Research Support from: This work was supported in part by grants from The Netherlands Organization for Scientific Research (Mozaiek grant), J. Adelmeijer: None Declared, R. Porte: None Declared, T. Lisman Grant/Research Support from: This work was supported in part by grants from The Netherlands Organization for Scientific Research (VIDI, 917.11.304).

PO003-MON

The novel c.774_775 ins ccgcgcc mutation combined with c.4085 T>A in the ADAMTS13 gene contributes to thrombotic thrombocytopenic purpura (TTP)Paiva J¹, Kempfer AC², Woods AI², Casinelli MM¹, Sanchez-Luceros A^{1,2} and Lazzari MA²¹Hemostasia y Trombosis, Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina; ²Laboratorio de Hemostasia y Trombosis, IMEX-CONICET, Academia Nacional de Medicina, Ciudad Autónoma de Buenos Aires, Argentina**Background:** PTT is a rare disease characterized by widespread intravascular platelet thrombosis.**Aims:** We described a case of congenital TTP with presence of a known (c.4085 T>A) and a novel (c.774_775 ins ccgcgcc) mutation, both in heterozygous state.**Methods:** The proband (P) is a male patient diagnosed TTP at the age of 37, with manifestations of thrombocytopenia and impaired renal function. ADAMTS13 activity (normal value: 40–130%), antigen (0.60–1.60 µg mL⁻¹) and IgG anti-ADAMTS13 antibody (< 15U mL⁻¹) were evaluated by ELISA and ultralarge von Willebrand factor (ULVWF) multimers (≤ 15%), by SDS-agarose gel electrophoresis. All exons were sequenced using ABI Prism 310 and *in silico* analysis by ORF Finder and TRANSLATE TOOL. Informed consent and ethical approval were obtained.

Results: P: ADAMTS13 activity = 0%, antigen = 0 $\mu\text{g mL}^{-1}$, IgG anti-ADAMTS13 antibody = 8 U mL^{-1} , ULVWF multimers = 58% and c.4085T>A known mutation, SNP c.-169C>T in region 5'UTR and novel mutation c.774_775ins ccgcgcc, all in heterozygous state.

Father and sister: ADAMTS13 activity = 46% and 56% and antigen = 0.67 and 0.90 $\mu\text{g mL}^{-1}$ respectively; both of them IgG anti-ADAMTS13 antibody = 8 U mL^{-1} .

Both, heterozygous for c.774_775ins ccgcgcc, presented normal ULVWF multimers. None showed ICs. *In silico* for the new mutation predicted the formation of a premature stop codon during transcription (p.G258PfsX133).

Conclusion: We observed (Calderazzo 2012) that c.4085A>T mutation causing ADAMTS13 intracellular retention is accompanied by c.-169C>T in several patients ($n = 4$). It is described that SNPs or mutations at 5'UTR region may affect transcription and translation of proteins.

The reading frame shift caused by c.774_775ins ccgcgcc also produced 177 different amino acids prior to the stop codon and has the distinctive feature of being, in turn, a doubling. Pimanda2004 and Garagiola2008 concluded that the insertions are associated with low ADAMTS13 activity. Therefore, the compound heterozygous state would justify low levels of ADAMTS13 in the P.

Disclosure of Interest: None declared.

PO004-MON

Thrombotic thrombocytopenic purpura (TTP) in a member of a family with ADAMTS13 gene abnormalities

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Background: TTP is characterized by thrombocytopenic purpura, microangiopathic hemolysis and microvascular thrombosis.

Aims: We describe a congenital TTP with the presence of one known (c.4085A>T) and two new mutations (c.2610 + 5G>C, c.2677C>G).

Methods: Patient (P) presented recurrent episodes of thrombocytopenia, headache, jaundice and abdominal pain since 14 years old. von Willebrand factor antigen (VWF:Ag; normal value: 50–150%) was assessed by ELISA as well as ADAMTS13 activity (40–130%), antigen (0.60–1.60 $\mu\text{g mL}^{-1}$), IgG anti-ADAMTS13 antibody (< 15 U mL^{-1}), detection of ADAMTS13-immune complexes (ICs) and anticardiolipin antibodies (ACA); genetic analysis using ABI Prism. The mutations were analyzed by *in silico* tools to predict their effect. Informed consent and ethical approval were obtained.

Results: P's had VWF:Ag = 158%, ADAMTS13 activity = 2.1%, antigen = 0.02 $\mu\text{g mL}^{-1}$ and IgG anti-ADAMTS13 antibody = 4.3 U mL^{-1} . Parents and siblings had normal VWF:Ag and ADAMTS13 activity and antigen, but the sister showed 27 U mL^{-1} of IgG anti-ADAMTS13 antibody. None of them was positive for ACA or ICs. The genetic analysis revealed 3 mutations: c.2610 + 5G>C in exon 20, c.2677C>G in exon 21 from the father and c.4085A>T in exon 29 accompanied by c.-169C>T from the mother. Only the P had both the mutation of the father and of the mother in heterozygous state. The sister and one brother only had the father's mutation, and the other brother had no mutations at all.

Conclusion: Future studies of the P's sister will probably show whether the presence of the said antibodies precedes clinical onset in TTP. We already knew that c.4085A>T (p.D1362V) causes intracellular retention of ADAMTS13 (Calderazzo2012). *In silico* predicted that c.2610 + 5G>C causes the loss of the splice donor site with a gain of 43 amino acids before encountering a stop codon in retained intron 20.

Simulation for c.2677C>G (p.A893P), predicted that it would be a tolerable change.

Compound heterozygous state would justify the phenotype in the P.

Disclosure of Interest: None declared.

PO005-MON

Congenital thrombotic thrombocytopenic purpura (TTP) caused by compound heterozygosity of c.1225C>T and a novel c.2321delG ADAMTS13 gene mutations

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Background: Severe deficiency of ADAMTS13 may result in von Willebrand factor ultralarge multimers (ULVWF) spontaneously interacts with platelet receptors, resulting in recurrent attacks of microvascular thrombosis.

Aims: We hypothesized that a compound heterozygous for both a known mutation (c.1225C>T) and a novel mutation (c.2321delG) may confer a more severe TTP phenotype.

Methods: Patient (P) presenting purpura and jaundice, with hemolytic anemia, thrombocytopenia and reticulocytosis at birth followed by several episodes during her first year of life. ADAMTS13 activity (normal value = 40–130%), antigen (0.60–1.60 $\mu\text{g mL}^{-1}$), and IgG anti-ADAMTS13 antibody (< 15 U mL^{-1}) were assayed by ELISA kits. ULVWF ($\leq 15\%$) was evaluated by SDS-agarose gel electrophoresis and genotyping was performed by sequencing on the ABI Prism 310. Mutations were analyzed by *in silico* tools to predict their effect. Informed consent and ethical approval were obtained.

Results: The P had undetectable activity of ADAMTS13, antigen: 0.04 $\mu\text{g mL}^{-1}$, IgG anti-ADAMTS13 antibody = 5 U mL^{-1} and ULVWF = 53%. The father and the mother showed no clinical manifestations and ADAMTS13 activity values of 53% and 52% respectively, IgG anti-ADAMTS13 antibody = 2 U mL^{-1} and normal ULVWF while antigen was of 0.58 and 0.65 $\mu\text{g mL}^{-1}$ respectively. Two mutations were found in P c.1225C>T inherited from the father and a novel mutation c.2321delG inherited from the mother.

Conclusion: The c.1225C>T (p.R409W) mutation affects the TPS1 domain, abolishing ADAMTS13 secretion. In a family with P in homozygous state to this mutation found by Camilleri 2012, the parents, in heterozygous state, showed normal activity and subnormal levels of antigen, like our P's father. On the other hand, *in silico* tools predicted the novel mutation c.2321delG (p.G774AfsX3) produced the reading frameshift with formation of a premature stop codon which would result in a nonfunctional protein.

We conclude that the genotype of P, due to compound heterozygosity of two mutations, would produce the TTP phenotype.

Disclosure of Interest: None declared.

PO006-MON

Quality assessment for ADAMTS-13 testing: a Canadian experience

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Background: Clinical testing of plasma ADAMTS-13 activity and inhibitors has become instrumental to distinguish and monitor congenital or acquired thrombotic thrombocytopenic purpura from other forms of thrombotic microangiopathies including atypical hemolytic uremic syndrome.

Aims: Evaluate the inter-laboratory variations for ADAMTS-13 activity and antibody measurements in Canada, propose recommendations for testing, data reporting and interpretation of the results.

Methods: Eight laboratories across Canada participated in the survey. Three series of 4 samples were sent from 1 site to the other sites for blinded measurement of ADAMTS-13 activity and anti-ADAMTS-13 antibodies. For activity testing, 6 sites used the Technozym[®] ADAMTS-13 activity ELISA assay (Technoclone) and 1 site used a FRET-S-VWF73 substrate-based fluorescent assay (peptide International Inc.). 2 sites used ELISA antigenic assays (R&D Systems and Sekisui), in addition to, or instead of, activity assays. For antibodies testing, 5 sites used the Technozym[®] ADAMTS-13 INH ELISA assay (Technoclone), 1 site used the IMUBIND[®] ADAMTS13 Autoantibody ELISA assay (Sekisui) and 1 site performed an in-house titration ELISA assay using recombinant ADAMTS-13 (Baxter) and serial plasma dilutions.

Results: There was a relatively good agreement between laboratories for ADAMTS-13 activity, in particular for low ADAMTS-13 activity levels. Results were reported as Units per mL or as percentage of normal plasma activity. Values below 10% activity were reported using the cutoff '< 10%' or using the actual value. There was also a relatively good agreement between laboratories for antibody detection. Results were either reported as Units per mL or as a titer. There was wide variability in reporting comments by each laboratory.

Conclusion: While overall laboratories performed well in detecting low levels of ADAMTS-13, there is a need for harmonizing reporting units and comments for both ADAMTS-13 activity and antibodies for which we propose a series of recommendations.

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PO007-MON

Portal vein thrombosis in liver cirrhosis: the role of ADAMTS13

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Background: Portal vein thrombosis (PVT) is a rare complication that dramatically changes the prognosis of cirrhotic patients especially those waiting for allogeneic liver transplantation. However, the possible contribution of von Willebrand factor (VWF) and ADAMTS13 in PVT is scarcely documented.

Aims: We evaluated the prevalence of PVT in cirrhotic patients as a function of (a) classical thrombophilic risk factors; (b) alterations of VWF and ADAMTS13 levels.

Methods: This study enrolled 60 cirrhotic patients > 18 year old referring to the Liver Unit of Gemelli Hospital during 2013 with liver cir-

rhosis of any etiology, diagnosed using sonographic and/or histological criteria. The only exclusion criterion was the presence of a neoplasm.

Results: The 60 enrolled patients were divided according to the ultrasound evidence of PVT. The group without PVT (group A) included 47 patients (m:f = 30:17) with different cirrhosis etiology (24 virus-related; 15 alcohol-related; 8 other causes). Their Child-Pugh scores were A (57.4%) and B-C (42.6%). The PVT group (group B) included 13 patients (m:f = 8:5) with different etiologies (5 virus-related; 6 alcohol-related; 2 other causes) and Child-Pugh scores A (23.1%) and B-C (76.9%).

Mann-Whitney analysis showed not significant differences of the Child-Pugh score between group A and B, while ADAMTS13 activity results significantly lower in group B (Median [IQR], 67.45 [41.11–88.00]; 9.98 [1.67–16.98], $P < 0.0001$). Interestingly, there are no statistical differences in the levels of VWF (antigen, RCo and RCo/Ag), FVIII and classical thrombophilic factors between the two groups. Furthermore, no correlation was found ($P = 0.326$) between the Child-Pugh score and ADAMTS13 activity, suggesting that the reduction of enzyme level is not proportional to the loss of liver parenchyma mass.

Conclusion: The obtained results show that ADAMTS13 can be considered an early independent risk factor for the development of PVT.

Disclosure of Interest: None declared.

PO008-MON

Importance of monitoring activity and anti-ADAMTS-13 antibodies levels in pregnancy with severe preeclampsia history

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Background: ADAMTS-13 – plasma metalloprotease capable of cleaving a specific Tyr-Met bond in the A2 domain of von Willebrand factor (VWF). Increased synthesis During pregnancy synthesis of VWF increases while activity of ADAMTS13 reduces.

Aims: In this study, we tried to establish a link between the presence of severe PE in history and a possible reduction in the activity of ADAMTS-13 or the presence of antibodies to this metalloproteinases.

Methods: We analyzed the 3 cases of severe PE in history with oliguria/anuria, thrombotic complications and/or multiorgan failure. The study was conducted 6–12 months after undergoing PE. Laboratory Methods

determination of genetic thrombophilia, homocysteine levels, APA, ADAMTS-13 activity and anti-ADAMTS-13 antibodies.

Results: In all patients outside of pregnancy levels of ADAMTS-13 activity is was not elevated while the antibody titers was increased (28.5 U mL⁻¹; 57.0 U mL⁻¹ and 145 U mL⁻¹). In one patient homocysteine levels was increase up to 27 mmol L⁻¹. None of the patients was not found thrombophilia. The patient with the level of anti-ADAMTS-13 145 U mL⁻¹ had history of severe PE, AFD, pulmonary embolism and 3 cases of ischemic strokes, which were regarded by neurologists as Sneddon syndrome. In subsequent pregnancy with a diagnosis of 'seronegative APS' patient received LMWH, she was delivery at 38 weeks due to presented of emerging signs of intrauterine fetal distress, PE did not develop. 2 years later, she became pregnant again: from 32 weeks occurred increase of ADAMTS-13 to 180 U mL⁻¹. She

was delivered at 37 weeks – Cesarean section due to decline fetal biophysical profile. PE has not developed.

Conclusion: Elevated levels of anti-ADAMTS-13 antibodies may be the cause of severe PE. Control antibodies to ADAMTS-13 has a predictive value and allows to adjust anticoagulant therapy and to determine the time of delivery, taking into account the risks to the mother and fetus.

Disclosure of Interest: None declared.

Angiogenesis / vasculogenesis

PO009-MON

PAR1-stimulated platelets enhance angiogenic activities of endothelial progenitor cells more potently than PAR4-stimulated platelets

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Background: Cardiovasculature maintains endothelial homeostasis by proliferation of adjacent endothelial cells (ECs) and recruiting circulating endothelial progenitor cells (EPCs). Platelets are closely involved in angiogenesis, apart from their primary functions in thrombosis and haemostasis. Platelets store both pro- and anti-angiogenic regulators, which are selectively released upon stimulation by thrombin receptor PAR1 and PAR4, respectively. The latter may be an important regulatory machinery of vessel regeneration and remodelling.

Aims: To investigate if PAR1-stimulated platelet releasate (PAR1-PR) and PAR4-PR differently regulate angiogenic properties of EPCs.

Methods: EPCs were generated from peripheral mononuclear cell culture. Washed platelets ($2 \times 10^9 \text{ mL}^{-1}$) were stimulated by PAR1-activating peptide (PAR1-AP; 10 μM) or PAR4-AP (100 μM) to prepare PAR1-PR and PAR4-PR, respectively.

Results: PAR1-PR or PAR4-PR had little influence on EPC proliferation. EPC migration experiments using a modified Boyden chamber showed that both platelet releasates facilitated EPC migration. As for *in vitro* tube formation on matrigel, PAR1-PR and PAR4-PR similarly enhanced capillary-like network formation of EPCs in the complete EPC medium containing 10% FBS and a cocktail of growth factors, whilst PAR1-PR more profoundly increased EPC tube formation in basal culture medium supplemented with only 0.5% FBS than PAR4-PR. The latter was confirmed in the murine angiogenesis model of subcutaneous matrigel implantation. Moreover, blockade of VEGF, SDF-1 α , or MMPs attenuated EPC migration and tube formation, suggesting a cooperation of these factors in the enhancements.

Conclusion: PAR1-PR enhances vasculogenesis more potently than PAR4-PR, and the enhancements require a cooperation of multiple platelet-derived angiogenic regulators.

Disclosure of Interest: None declared.

PO010-MON

Development of an *ex ovo* chicken embryo model for measuring traction forces during *in vivo* angiogenesis

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Background: Angiogenesis, which is the process of new blood vessel growth, plays a key role in tissue development, wound repair, and vari-

ous pathological states. During angiogenesis, endothelial cells interact with and deform the extracellular matrix; however, much of the mechanics and forces mediating this process *in vivo* are unknown.

Aims: To develop an *ex ovo* chicken embryo model that utilizes the highly vascularized chorioallantoic membrane (CAM) in conjunction with fluorescent scaffolds for measuring traction forces generated by migrating angiogenic endothelial cells.

Methods: Fertilized *Gallus gallus* eggs were incubated for 3 days. On day 3, eggs were broken and embryos were transferred to petri dishes. Polylactic acid scaffolds with known elastic properties were added to the CAM. Scaffolds contained 0.1% FITC or 0.1%, 0.01%, or 0.001% rhodamine and were cut into 2×2 , 1×1 , or 1×0.5 cm rectangles in a planar or rolled orientation. Embryos were injected via a CAM vein with 10 kDa Cascade Blue-dextran, 2MDa FITC-dextran, or FITC-lectin. To secure the scaffold to minimize movement during imaging, embryos were placed in a custom 3D printed chamber. To quantify traction forces, 3D time-lapse images of blood vessel growth into scaffolds were acquired using multi-photon microscopy. Embryos were sacrificed at day 13 and scaffolds were cryosectioned, stained, and imaged via confocal microscopy.

Results: The use of FITC-lectin and 2MDa dextran resulted in the greatest vessel fluorescence while the use of 0.001% rhodamine yielded the optimal scaffold fluorescence for multi-photon imaging. Injection of dextran increased embryo survival by 83% compared with lectin injection. Confocal imaging revealed maximal blood vessel interactions for 1×0.5 cm scaffolds in the rolled orientation.

Conclusion: Use of this model will further understanding of the fundamental biophysical mechanisms of *in vivo* angiogenesis, possibly providing a foundation for the development of new medical interventions for treating angiogenesis-associated diseases.

Disclosure of Interest: None declared.

PO011-MON

Microrna-126 reduces blood-retinal barrier breakdown in oxygen-induced retinopathy mouse model

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Background: Retinopathy is a sort of disease associated with retinal vascular dysfunction. Many reports have identified that microRNAs act as critical post-transcriptional factors on regulating their target genes and playing important roles in their functions in eye diseases. Our publication suggested that microRNA-126 level was lower in retinas of oxygen-induced retinopathy (OIR) than control mice. However, it is unknown that microRNA-126 is crucial for blood-retinal barrier (BRB) or not.

Aims: To evaluate the roles of miR-126 in regulating BRB function in OIR mouse model.

Methods: We established OIR mouse model and measured the levels of miR-126 in retinas with real-time PCR. After plasmid pCMV-MIR-126 was injected to vitreous, VCAM-1 levels in retina were detected by Enzyme-linked immunosorbent assay, and BCL2L11 were assessed by real-time RT-PCR. Retinal vaso-obliteration was detected by Fluorescein angiography. Immuno-histochemistry was used for detecting VCAM-1 expression or Albumin leakage in retinas. Albumin leaked in retinas was detected by immunoblotting. The apoptosis of retinal endothelial cells (RECs) was assessed by TUNEL Histology. The blood-retinal vascular leakage was evaluated using Evans-blue dye assay.

Results: Our results suggested that miR-126 was significantly decreased in retinas from OIR mice. Restoration of miR-126 in retinas overcame over-expression of VCAM-1 and BCL2L11, and then reduced retinal vaso-obliteration area, retinal vascular leakage, RECs apoptosis or retinal permeability in OIR model.

Conclusion: It explored that miR-126 could reduce blood-retinal barrier dysfunction via regulating VCAM-1 and BCL2L1 expression in OIR model.

Disclosure of Interest: None declared.

PO012-MON

Stimulation of PAR-1 or PAR-4 promotes a similar pattern of platelet-mediated angiogenesis

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Background: Platelets mediate vessel formation through the secretion of angiogenic-modulating factors from α -granules, including the pro-angiogenic vascular endothelial growth factor (VEGF) and the anti-angiogenic endostatin. Initial findings indicated that these molecules are packed into different α -granules and that they are selectively released by the specific stimulation of protease-activated receptor (PAR)-1 or PAR-4 using either platelet rich plasma (PRP) or washed platelets (WPs). In contrast, recent evidences are against this hypothesis.

Aims: To clarify these controversies, here we evaluated the release of VEGF and endostatin from WPs and PRP activated with PAR-1 activated peptide (AP) or PAR-4-AP and determined the angiogenic effect of platelet-releasates.

Methods: The levels of VEGF and endostatin (ELISA) and endothelial proliferation (enzymatic activity), wound healing (microscopy) and tubule formation (microscopy) were determined using supernatants from PAR-1- or PAR-4-stimulated platelets.

Results: We found that due to the high levels of plasmatic endostatin vs. platelet-lysates content (83 ± 5 vs. 2.5 ± 0.3 ng mL⁻¹), the release of endostatin induced by PARs activation was not detected using PRP. In contrast, PAR-1 or PAR-4 platelet activation resulted in a significant release of VEGF (10 ± 2 and 11 ± 2 ng mL⁻¹ vs. resting platelets $P < 0.001$) and the intra-platelet VEGF level was 10-fold higher than that of plasma (12 ± 1 vs. 1.3 ± 0.2 ng mL⁻¹). The activation of WPs with PAR-1 or PAR-4 AP promoted the secretion of VEGF and endostatin in a concentration-dependent manner being PAR-1 11–15-fold potent than PAR-4. Moreover, PAR-1- or PAR-4-stimulated WPs triggered endothelial cell proliferation, wound healing and tubule formation.

Conclusion: Our results support the notion that while circulating endostatin accounts for the maintenance of a systemic antiangiogenic state, locally, the release of platelet α -granule content mediated by PAR-1 or PAR-4 activation promotes angiogenesis.

Disclosure of Interest: None declared.

PO013-MON

Platelet membrane glycoprotein CD151 promotes endothelial progenitor cell angiogenesis

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Background: Platelets play an important role in angiogenesis, and promote angiogenesis of endothelial progenitor cells (EPCs). Understanding of the mechanisms of platelet angiogenic activities are, however, still modest.

Aims: To investigate if platelets can regulate angiogenic property of EPCs via mechanisms beyond platelet-released angiogenic regulators.

Methods: EPCs were generated by EPC-directed cell culture of peripheral mononuclear cells. Capillary-like tube formation of EPCs was assessed using an *in vitro* Matrigel assay.

Results: Platelets promoted EPC tube formation in both basic and complete EPC medium. The enhancement by platelets was more marked than by platelet releasates. Thus, platelets increased branching points of EPC tube formation from 30.5 ± 9.0 per field of EPCs alone to 95.5 ± 17.6 per field ($P < 0.01$; $n = 4$), which were higher than those by platelet releasates (58.2 ± 8.3 per field; $P < 0.05$ vs. platelets), indicating that platelet membrane components also promoted EPC tube formation. The latter was supported by that fixed platelets also enhanced EPC tube formation. Further work revealed that the enhancement involved platelet-expressed glycoproteins, because pre-treatment of platelets with neuraminidase, which removes sialic acid from platelet membrane glycoproteins, abolished the enhancement. Moreover, platelet-expressed CD151 accounted for the enhancement, as pre-treatment of platelets, but not EPCs, with the CD151 blocking antibody (clone 50-6) reduced the enhancement. Platelet CD151 promoted the tube formation via Src-PI3K signaling pathway of EPCs. Hence, platelet-promoted tube formation was almost abolished when the Src-PI3K signaling pathway of EPCs was blocked by the Src-specific inhibitor PP2 and PI3K-specific inhibitor LY294002.

Conclusion: Platelets enhance EPC angiogenesis through membrane-expressed glycoproteins, apart from platelet-released angiogenic regulators. The enhancement is exerted via CD151-mediated Src-PI3K signaling of EPCs.

Disclosure of Interest: None declared.

PO014-MON

Proangiogenic properties of fucoidan is associated with recruitment and modulation of macrophage polarization

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Background: Cardiovascular diseases such as Peripheral Arterial Disease may evolve towards critical limb ischemia (CLI), requiring revascularization or amputation. Fucoidan, a marine sulphated polysaccharide has shown promising angiogenic properties *in vivo*. Intramuscular injections of fucoidan in a mouse CLI model lead to protection of ischemic tissue against necrosis.

Aims: Since macrophages plasticity is known to play a key role in tissue remodelling after injury, we hypothesized that the improved healing is a result of a shift in macrophage polarization M1/M2 induced by fucoidan.

Methods: Perfusion and Boyden chamber assays allowed to study fucoidan treatment on *ex vivo* monocytes adhesion and extravasation on activated endothelium. The effect on macrophage polarization was evaluated *in vitro*, on human monocytes cultured with or without fucoidan in the presence of IFN- γ /M1 and IL4/M2 and characterized by examining the expression of specific M1/M2 markers by flow cytometry. The angiogenic capacity of their secretomes was studied on endothelial progenitor cells by wound healing and Matrigel assays. Mouse CLI model enabled us to probe the role of fucoidan treatment during early (2 and 4 days) and late (14 days) stages disease on macrophage recruitment and polarization in ischemic gastrocnemius.

Results: Monocytes stimulation with fucoidan resulted in a rapid increase in cell adhesion to activated endothelium in flow conditions and enhanced migration (3 and 5 times respectively $P < 0.05$). Fucoidan promotes macrophage polarization to M2-like subtype, increasing the proangiogenic potential of pro-angiogenic cytokines. *In vivo* fucoidan

dan recruits 8 times more CD45⁺Ly6G⁺ at day 2 and twice the CD45⁺F4/80⁺ at day 4 in ischemic gastronemius compared to control, leading to accelerate M2 polarization. These results correlate with the protective effect on muscle observed at day 14.

Conclusion: Our data shed new light on mechanisms by which fucoidan stimulate muscle regeneration.

Disclosure of Interest: None declared.

PO015-MON

Unaltered angiogenesis-regulatory activities of platelets from mild type 2 DM patients despite a marked platelet hyperreactivity

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Background: Type 2 diabetes mellitus (T2DM) is linked to platelet dysfunction and impaired tissue regeneration. Platelets store and release various angiogenic regulators that regulate angiogenesis and tissue regeneration.

Aims: To investigate possible alternations of platelet angiogenic regulator release and platelet-regulated angiogenesis in T2DM.

Methods: Twelve T2DM patients (61 ± 3 years; BMI 27.6 ± 0.9 ; blood glucose 8.6 ± 0.7 mM; HbA1c $6.4 \pm 0.3\%$) and matched non-diabetic controls were studied. Platelet reactivity was monitored by flow cytometry. Endothelial cell (EC) tube formation was assayed on matrigel-covered plates.

Results: Basal platelet activation was similar between DM and control subjects. However, the thrombin receptor activating peptide (TRAP; $4 \mu\text{M}$) induced a higher platelet P-selectin expression in DM ($35.5 \pm 9.2\%$ vs. $21.3 \pm 5.6\%$ in control; $P < 0.05$). Dynamic platelet reactivity (to $10 \mu\text{M}$ TRAP; assessed by platelet annexin V binding) was also enhanced in DM, i.e. a quicker surge of annexin V⁺ platelets ($21.5 \pm 5.7\%$ vs. $16.8 \pm 3.4\%$ in control at 2 min) and a sustained elevation during 8 min. Platelet released pro-angiogenic PDGF BB and VEGF and anti-angiogenic thrombospondin-1 and platelet factor 4 were measured by ELISAs. TRAP induced marked releases of platelet angiogenic regulators. The releases were, however, similar between two groups. Thus, TRAP increased VEGF from $66.9 \pm 29.5 \text{ pg mL}^{-1}$ at basal to $466.5 \pm 166.4 \text{ pg mL}^{-1}$ in DM and from 58.7 ± 38.9 to $465.5 \pm 229.3 \text{ pg mL}^{-1}$ in the controls. Platelet-enhanced capillary-like EC tube formation did not differ either. Thus, platelet supplementation (EC:platelet ratio 1:150) led to ~140% enhancements by platelets from both DM and control subjects, as assessed by the increases of the branch points of EC tube formation.

Conclusion: Platelets are hyperreactive in T2DM. Platelet angiogenic regulator release and platelet-enhanced angiogenesis are, however, similar between mild T2DM and control subjects.

Disclosure of Interest: None declared.

PO016-MON

Human platelet lysate gel (HPLG) supports and stimulates endothelial progenitor cell-driven vasculogenesis

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Background: Endothelial progenitor cells (EPCs) are circulating stem-like cells able to differentiate into mature endothelial cells and replenish the endothelial lining at the sites of vascular damage. Their utilization for cell therapies aiming to restore healthy endothelial lining of

blood vessels and stimulate neovascularization of ischemic tissues has been the object of intense investigation.

Aims: In this study, we investigated the use of human platelet lysate gel (hPLG) as an animal product-free and patient-specific tool to isolate, amplify, differentiate and deliver endothelial progenitor cells.

Methods: Anticoagulated human plasma was used to obtain hPLG by sonication in the presence of calcium. Endothelial colony-forming cells (ECFCs) were obtained from human peripheral blood. Colony formation rate (i.e. number of colonies per mL of peripheral blood), ECFC adhesion/migration rate, proliferation/viability, endothelial marker expression, substrate invasion and capillary network formation in 3D cultures were analyzed on hPLG and compared with the same parameters on collagen type I, which is currently used as a substrate for ECFC culture

Results: In our experiments, hPLG appeared to be a superior substrate compared to collagen I for ECFC isolation (higher colony count), amplification (higher viability and proliferation rate) and differentiation (expression of higher levels of VE-cadherin, vascular endothelial growth factor receptor 2 and von Willebrand Factor). In addition, hPLG appeared to stimulate the vasculogenic activity of ECFCs *in vitro*, as measured by the formation of an extended microvascular network *in vitro* when cells were embedded in hPLG but not when embedded in collagen gels or Matrigel™.

Conclusion: We propose the use of hPLG for ECFC isolation/delivery and the stimulation of tissue revascularization for therapeutic purposes.

Disclosure of Interest: None declared.

PO017-MON

TF-mediated positive feedback crosstalk between endothelial cells and patrolling CD14⁺/CD16⁺ monocytes stimulates angiogenesis

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Background: Angiogenesis after ischemia contributes to reduce organ damage and preserve cell survival. We recently reported monocyte (Mo)-endothelial cell (ECs) crosstalk mediated by Wnt5-Fz5 to induce EC-tissue factor (TF) expression and promote angiogenesis. However, the tentative role of inflammatory CD14⁺/CD16⁺ and patrolling CD14⁺/CD16⁺ monocytes is not known.

Aims: Here our objective was to investigate whether TF (as alternative spliced [asTF] or as TF-rich-microparticles [TF-MPs]) can influence monocyte polarization and signal ECs to form neovessels.

Methods: Angiotube formation was investigated using human microvascular-ECs (mECs) and human Mo-subsets. TF expression in mECs was determined by qPCR and immunoblotting. TF-MPs were analyzed by FACS. Full length TF (fTF) or as TF were over-expressed in mECs. Changes in CD16 and VE-Cadherin expression in monocytes were analyzed by FACS and vWF by immunofluorescence.

Results: Mo-CD14⁺/CD16⁺, but not Mo-CD14⁺/CD16⁻, significantly induced mECs to express fTF and asTF, to trigger Ets-1/CCL2 signaling, and to form angiotubes. ECs co-cultured with Mo-CD14⁺/CD16⁺ increased TF-MPs secretion. Compared to supernatants obtained from ECs not expressing TF, supernatants obtained from fTF as well as TF overexpressing ECs promoted Mo-CD14⁺/CD16⁻ polarization into Mo-CD14⁺/CD16⁺. Mo-CD14⁺/CD16⁺ differentiated into EC-like cells, identified by expression of the endothelial markers VE-Cadherin and vWF.

Conclusion: Mo-CD14⁺/CD16⁺ induce mEC to express TF and form angiotubes. TF released by ECs induce Mo-CD14⁺/CD16⁻ polarization

tion into Mo-CD14⁺/CD16⁺, and further to differentiate into EC-like cells, favoring microvessel formation. These results show a TF-mediated positive feedback crosstalk between endothelial cells and monocytes to stimulate angiogenesis.

Disclosure of Interest: None declared.

PO019-MON

PAR2-dependent signaling via SMAD3 in TF-induced angiogenesis

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Background: Tissue factor (TF) signaling regulates gene expression and protein synthesis leading to the modulation of cell function. Recently, we demonstrated that in microvascular endothelial cells (mECs) TF signaling induces activation of ETS1 that induces CCL2 secretion stimulating smooth muscle cell recruitment and stabilization of neovessels. Because combinatorial control is a characteristic property of ETS family members, we hypothesized that such an interaction could also be possible in TF signaling through ETS1.

Aims: Here we have investigated whether additional transcription factors are involved in TF-induced mature microvessel formation.

Methods: We used human microvascular endothelial cells and a murine hemangioma cell line (EOMA) to perform *in vitro*-3DBM and *in vivo*-matrigel implants experiments.

Results: Data show that in addition to ETS1, SMAD3 contributes to tube-like stabilization induced by TF. Moreover, while the signaling TF/AKT/ETS1 pathway is PAR2 independent, PAR2 is required for TF/SMAD3 signaling. PAR2-dependent activation of SMAD3 is mediated by PKC phosphorylation and independent of TGF- β . In addition, disruption of SMAD3 expression in mECs reduces ERK1/2 phosphorylation and decreases target gene promoter activity, reducing CCL2 expression and angiotube stabilization. EOMA cells that do not express TF but express PAR2, proliferate in disorganized cell clusters; transfection of TF into EOMA cells induces angiotube formation *in vitro*, and a well organized microvessel formation within the hemangioma reducing its size *in vivo*. EOMA-TF⁺ cells showed an increased ETS1 and SMAD3 expression.

Conclusion: TF-induced angiogenesis results from two cooperative pathways: TF-induced microvessel formation through β 1-integrin/AKT1/ETS1 and TF-induced microvessel stabilization through PAR2/SMAD3. Angiogenesis is a complex process consequence of a SMAD3/ETS1 transcriptional cooperation in response to TF signaling in mECs.

Disclosure of Interest: None declared.

PO020-MON

Increase of reactive oxygen species and senescence down the proliferative rate of endothelial progenitor cells from patients with venous thromboembolic disease

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Background: Endothelial progenitor cells (EPC) were described and soon after endothelial colony-forming cells (ECFCs) were demonstrated. Our work group reported that ECFCs from patients with venous thromboembolism (VT) show a dysfunctional state.

Aims: We investigated the potential of proliferation of long-life ECFCs associated with the presence of reactive oxygen species and senescence in patients with VT.

Methods: Mononuclear cells from peripheral blood of patients with VT and blood donors (control) were obtained and ECFCs were identified by flow cytometry. ECFC-derived endothelial cells (ECs) from patients VT and controls were serially passaged to determine their proliferative potential obtained over 120 days. Senescence was evaluated by β -galactosidase reaction and reactive oxygen species (ROS) analyzed by flow cytometer. RNA isolated from ECFC-derived ECs was evaluated by RT-PCR expression of ephrin 4 and ephrin 2. The study protocol was reviewed and approved by the Human Ethical Committee of the IMSS, and conforms to the guidelines of the 1975 Declaration of Helsinki. Informed written consent was obtained from all subjects before enrollment.

Results: None ECFC-derived ECs expressed antigens of hematopoietic or mesenchymal cells however both groups expressed antigens of endothelial cells. The ability to proliferate in control cells was increased and they could be expanded for at least 35 population doublings (PDs). In contrast, proliferation was reduced in ECFC-derived EC from patients with VT because they were passaged only during 20 PDs with an increased senescence as evaluated from VT patients as compared with those obtained from control. Ephrin 4 significantly was more expressed in ECFCs from VT patients vs. controls.

Conclusion: Our data suggest an abnormality in the mechanisms related with the cell cycle of ECFCs in VT patients resembling some characteristics of dysfunctional ECs like ROS production and senescence. These findings may help to understand some pathophysiological aspects of VT.

Disclosure of Interest: None declared.

Animal models I

PO021-MON

Plasmin, not thrombin, activates complement in arterial and venous thrombosis

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Background: Strong evidence indicates that there is cross-talk between complement and coagulation. This is highlighted by reports that thrombin (IIa) cleaves and activates complement C5. The physiologic relevance of this interaction has not been fully tested *in vitro* or in models of thrombosis.

Aims: To assess the efficiency of IIa, fXa and plasmin (Pn) in generating C3a and C5a *in vitro* and in murine models of arterial (AT) and venous thrombosis (VT).

Methods: C3a, C5a, and thrombin-antithrombin (TAT) were quantified by ELISA *in vitro* and in murine FeCl₃-induced AT and IVC-stasis induced VT. Some arterial thrombi were thrombolysed with a plasminogen (Pg) activator.

Results: In AT ($n = 33$), C3a correlated strongly with C5a ($r = 0.82$, $P < 0.0001$) but TAT did not ($r = -0.04$) suggesting that (complement) convertases, not IIa, generate C5a in AT. By contrast, C3a was poorly correlated with C5a in VT ($n = 14$; $r = -0.05$). Interestingly, clot weight (CW) was strongly correlated with C5a ($r = 0.78$, $P < 0.001$) in VT, suggesting that processes triggered during VT drive C5a generation. TAT and C5a were poorly correlated ($r = 0.47$, $P = 0.093$) in VT, suggesting that IIa does not directly activate C5 in VT. To establish a link between CW and C5a we used *in vitro* assays and found that the catalytic efficiency of C5a generation by Pn ($2.3 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$) far exceeded that of IIa or fXa, and was similar to that published for convertases. To characterize C5a generation by Pn *in vivo*, we thrombolysed arterial thrombi and observed a ~2-fold increase in C5a levels, suggesting that Pn generates C5a *in vivo*. C3a was increased by a similar margin despite being poorly cleaved by IIa, fXa and Pn *in vitro*. Unchallenged Pg^{-/-} mice had significantly lower levels of C5a, lending further support for Pn as a convertase.

Conclusion: Our data are consistent with Pn (or a downstream substrate) being a bona fide convertase during thrombosis *in vivo*. Future work will assess this interaction in human disease.

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PO022-MON

Establishment of a vascular injury model demonstrating different efficacies of antithrombotic agents on thrombus formation in mice

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Background: Several thrombosis models have been used to investigate the mechanism of thrombus formation in live animals and they each

have advantages and disadvantages that include non-pathophysiological injury and impractically complicated manipulation.

Aims: The objective of this study was to develop a well-controlled mouse model of mechanical injury that can be used to evaluate the efficacy of antithrombotic drugs in inhibiting thrombus formation *in vivo*.

Methods: The mechanical injury was exerted upon the abdominal aorta by pinching with hemostatic forceps for either 15 (moderate injury) or 60 (severe injury) seconds. Thrombus formation was monitored for 20 min in real time using a fluorescent microscope coupled to a CCD camera.

Results: In the moderate injury, the mean maximal size of the thrombi was $0.2954 \pm 0.01503 \text{ mm}^2$, and the mean AUC (area under the curve) was $165.2 \pm 17.29 \text{ mm}^2$ with one typical peak of thrombus size. Upon the severe injury, the mean maximal size of the thrombus was $0.7300 \pm 0.04043 \text{ mm}^2$ and AUC was $600.5 \pm 37.77 \text{ mm}^2$ with additional peaks of thrombus size. Interestingly, administration of clinical drugs inhibited the formation of thrombi to different extent. Aspirin reduced the thrombus size in the severe injury by 40% ($P = 0.0016$, $n = 5$) compared to untreated animals, but had no significant effect on the thrombus formation in the group upon the moderate injury ($P = 0.3623$, $n = 4$). Drugs targeting GP IIb-IIIa (eptifibatide), ADP receptor P₂Y₁ (MRS2500) and P₂Y₁₂ (clopidogrel), and thrombin (hirudin) alleviated thrombus size by 94.1%, 64.7%, 67.6% and 55.9%, respectively upon the moderate injury, while the thrombus size decreased by 79.7%, 62.7%, 66.1% and 49.1%, respectively in the severe injury.

Conclusion: We have established a simple vascular injury model with optimal reproducibility and feasibility that allows us to evaluate the efficacy of anti-thrombotic drugs.

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Disclosure of Interest: None declared.

PO023-MON

Ferric chloride-induced venous thrombosis model in rats: finding the optimal concentration of ferric chloride

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Background: Experimental animal models remain the essential component in studies of venous thrombosis. One of the models that are widely used in research is ferric chloride (FeCl₃)-induced thrombosis. However the protocols for the model vary greatly in terms of FeCl₃ concentrations applied, favouring higher (up to 60%) concentration that may lead to quick uncontrolled vessel occlusion resistant to anti-thrombotic agents.

Aims: This study was designed to determine the range of ferric chloride concentrations adequate for consistent vessel occlusion all the while sensitive to antithrombotics for better use of this model in both basic thrombosis research and screening of new and potential anticoagulants.

Methods: All procedures involving laboratory animals were approved by SPCPA Bioethical Committee. Outbred albino male rats (370–400 g, $n = 30$) were anesthetized (chloral hydrate 400 mg kg⁻¹ in saline, IP). Ferric chloride (FeCl₃) in different concentrations (from 2.5% to 20%) was applied directly to the previously exposed inferior vena cava (IVC) for 10 min to induce thrombosis. One minute prior to FeCl₃ application rats received either 100 U kg⁻¹ of heparin or 0.9% saline intravenously. Ultrasound contact gel was applied directly to the IVC. Thrombus formation was monitored by ultrasound system with 22 MHz transducer.

Results: Images of the IVC were obtained every 5 min (total time 75 min). Maximum surface area of the forming clot was measured in axial and transversal view. Heparin completely suppressed clot propagation induced by 2.5% FeCl₃, significantly inhibited it in groups where clotting was induced by 5% and 10% FeCl₃, but showed little affect on 10% FeCl₃ group and no influence on 20% FeCl₃-group compared to saline.

Conclusion: Our preliminary data suggests that the optimal range of ferric chloride concentration for modeling venous thrombosis in rats lies between 5% and 15%. The second series of experiments with smaller concentration intervals to narrow down and clarify the range is currently in progress.

Disclosure of Interest: None declared.

PO025-MON

Thrombin generation in zebrafish

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Background: Recently, whole blood (WB) calibrated automated thrombogram (CAT) was miniaturized enabling the measurement of thrombin generation (TG) in a limited volume (5 µL) of WB. Consequently, this approach may be used to determine TG in small lab animals. Zebrafish are readily available test animals for genetic research. However, their small size has been a hurdle in thrombosis and hemostasis research since most assays require large amounts of plasma.

Aims: To verify the possibility to measure TG in zebrafish using our newly developed miniaturized WB assay.

Methods: For TG, 5 µL of WB was mixed with 5 µL of buffer containing a rhodamin-based thrombin-sensitive P₂Rho substrate (final concentration (fc) 300 µM). 5 µL of this mixture was put on a paper disk and covered with mineral oil to prevent evaporation. Calibration was done as described (Ninivaggi *et al.* Clin Chem 2012) by adding 5 µL of WB to 5 µL of a mixture containing P₂Rho (fc 300 µM), a₂M-thrombin calibrator (fc 100 nM) and citrate (fc 9.8 mM). Fluorescence was detected with a fluorometer (570 nm).

Results: Due to their limited blood volume, it is impossible to perform both a TG and calibrator measurement on one fish. Since calibrator measurements performed on blood from different fish demonstrated variation < 20%, the average calibrator slope was used for calculations. TG measured in individual fish showed same amounts of inter-individual variation as in humans. Striking differences with human TG were the short lag time, high peak and high velocity index. Further analysis of the fibrin network of the clot, by means of scanning electron microscopy (SEM), showed a much denser network compared to humans.

Conclusion: These results demonstrate the feasibility of measuring TG in WB collected from zebrafish. Consequently, zebrafish may be used as a *in vivo* model to test the effect of (novel) therapeutics on TG and serve as a model organism for mechanistical research in thrombosis and haemostasis.

Disclosure of Interest: None declared.

PO026-MON

Clot contraction and polyhedrocytes in clots formed after saphenous vein puncture in the mouse

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Background: Little has been known about the structure and composition of contracted (retracted) blood clots or thrombi. During the contraction of blood clots *in vitro*, fibrin and platelets, initially homogeneously arranged throughout the clot, are redistributed primarily to the exterior, and erythrocytes on the interior are compressed to form a tightly packed array of polyhedra, called polyhedrocytes. Although polyhedrocytes have been found in some coronary artery thrombi, they are not a primary component.

Aims: The major objective was to study the composition of venous clots formed during vascular injury, and to quantify their components, including the presence of erythrocytes (normal, polyhedrocytes, intermediates), leukocytes, platelets, fibrin, and microparticles.

Methods: The saphenous vein of C57B6 mice was exposed and punctured with a 23G needle. The clots were dissected out together with the vessel, washed, fixed, and prepared for and examined by scanning electron microscopy.

Results: The images of these venous clots were quantified, accounting for all structures observed. We found that in the 18,348 structures quantified, polyhedrocytes and intermediate forms of compressed erythrocytes were a major component of these clots, making up about 43% of the volume, with only 2% biconcave erythrocytes. Other components were: fibrin, 40%; platelets, 10%; microparticles, 4%; leukocytes, 1%. Clots very commonly contained closely packed polyhedrocytes surrounded by fibrin and platelets, as observed in contracted clots *in vitro*.

Conclusion: These results demonstrate that polyhedrocytes are generated *in vivo* during hemostasis of large vein injury. Since polyhedrocytes surrounded by a meshwork of fibrin and platelets form a nearly impermeable barrier, they could physically limit blood loss, and stabilize the clots by preventing access to fibrinolytic enzymes. Therefore, polyhedrocytes could be used to assess bleeding or thrombotic risk and response to therapy.

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PO027-MON

Beneficial therapeutic properties of dietary alpha-linolenic acid (ALA) in a mouse model of sickle cell disease

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Background: Sickle cell disease (SCD) is a genetic disease characterized by complex manifestations and is associated with high morbidity and mortality. Small trials in patients have shown potentially promising results of long-chain omega-3 fatty acids.

Aims: Due to the risks and limited access related to the consumption of fishery products, we hypothesized that the plant-derived omega-3

ALA has therapeutic effects in SCD, since our earlier studies have indicated its anti-thrombotic, anti-inflammatory and anti-platelet effects.

Methods: Eight-week-old Berkeley mice were put on a diet with a low- (0.03%) or a high- (7.3%) ALA content for 4 weeks. Blood was analysed for reticulocytes and reticulated platelets, neutrophils/RBC/platelet aggregates, platelet activation, neutrophils PSGL-1, sickle cells. Endothelial VCAM-1, ICAM-1, P-selectin and vWF expression was evaluated in sections from aorta. Spleen, liver, lungs and kidney were stained with H/E and evaluated for necroses.

Results: Four-weeks feeding with a high-ALA diet did not change the % of reticulocytes nor reticulated platelets compared to the low-ALA group, but reduced the platelets/neutrophils aggregates (LA 83.5% vs. HA 67.6%, $n = 8$, $P = 0.03$). Platelet P-selectin at basal level was significantly reduced in the high-ALA mice (MFI: 288 LA vs. 86 HA, $n = 8$, $P = 0.01$), as well as activated integrin IIbIIIa (MFI: 298 LA vs. 90 HA, $P = 0.06$). Accordingly, PSGL-1 expression on neutrophils was also reduced by ALA (MFI: 3629 LA vs. 2189 HA, $n = 3$, $P = 0.01$). GpIb expression was higher on high-ALA platelets, confirming reduced activation (MFI: 884 LA vs. 1294 HA, $n = 8$, $P = 0.04$). Endothelial VCAM and ICAM were also significantly reduced in aortae from high-ALA mice (VCAM area: 1640 μm^2 LA vs. 658 HA, $P = 0.002$; ICAM: 3599 μm^2 LA vs. 1170 HA, $P = 0.01$).

Conclusion: Dietary ALA inhibits platelets, neutrophils and endothelial cell activation in a murine model of SCD, thus representing a possible ubiquitous and readily available therapeutic agent for sickle cell disease.

Disclosure of Interest: None declared.

PO028-MON

Rabbit model of ferric chloride-induced carotid arterial thrombosis optimized for translational drug discovery

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Background: Animal models of thrombosis are important tools in thrombosis research and drug discovery.

Aims: The purpose of this work is to establish a rabbit model of carotid arterial thrombosis suitable for drug discovery in comparison of electrical injury and/or ferric chloride (FeCl₃)-injury of the carotid artery.

Methods: Male New Zealand White rabbits were subjected to electrically or FeCl₃ induced carotid arterial thrombosis. A number of variables have been evaluated for electrical injury. A range of FeCl₃ concentrations (10–50%) has been assessed to demonstrate the consistent and complete occlusion of the carotid artery. Thrombus formation was measured by a Doppler blood flowmeter (quantitated by means of area under the curve) and validated by thrombus weight. Apixaban (a FXa inhibitor) and clopidogrel (a P2Y₁₂ antagonist) were applied to validate the utility of FeCl₃ injury as an efficacy model.

Results: Under the most optimal conditions of all the key variables for the electrically induced carotid arterial thrombosis, this model failed to deliver the consistent and complete occlusion of the vessel. In contrast, FeCl₃ injury model was more consistent and easy to operate after the optimization of FeCl₃ at 50% concentration. Under our refined conditions of FeCl₃ injury, both apixaban and clopidogrel dose-dependently inhibited arterial thrombus formation, with ED₅₀ of 0.09 mg kg⁻¹ per h, i.v. and 1.27 mg kg⁻¹, p.o. for apixaban and clopidogrel, respectively. Plasma drug exposure and activated partial thromboplastin time (aPTT) and prothrombin time (PT) assays were used to confirm drug effects for apixaban, and ADP-induced platelet aggregation for clopidogrel.

Conclusion: The present study demonstrated the technical challenge of the electrical injury thrombosis model and its inferior to the FeCl₃ injury model in rabbit, and showed that the feasibility of FeCl₃ injury as an efficacy model for translational drug discovery research.

Disclosure of Interest: None declared.

PO029-MON

Dissection of the therapeutic efficacy of fix-triple for reducing haemophilic arthropathy in hemophilia B using a blood-induced joint injury murine model

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Background: Bleeding into joints that consequently develops into haemophilic arthropathy (HA) is the most common morbidity of haemophilia, and impacts negatively on quality of life. Patients are treated with factor IX, a clotting factor essential for hemophilia B. Despite high quality plasma-derived and recombinant factor IX protein products having been readily available for several decades, many patients still suffer from HA. This suggests that the current FIX products are not protective against HA. We suspect that this may be due to the low trough level of the FIX products currently used. We previously demonstrated that the activity FIX-Triple (FIX-V86A/E277A/R338A) is superior to FIX-WT (wild-type) in gene therapy and in protein replacement therapy for hemophilia B. Moreover, we found that hemophilia B mice treated with FIX-Triple maintained 10 times higher basal activity than those treated with FIX-WT.

Aims: We aim to test whether FIX-Triple will be more effective than FIX-WT in the reduction of HA incidence.

Methods: Gene transfer with adeno-associated virus (AAV) was performed on hemophilia B mice. Joint injury was induced by inserting a 30 G needle into the right knee joint of mice to create the 'haemorrhage-induced joint injury'. Fourteen days after injury, mice were sacrificed and the parameters including appearance of joint, joint diameter, visual bleeding score (VBS), and microCT imaging were assessed.

Results: Mice treated with AAV-FIX-Triple showed 5.4-fold higher specific clotting activity than those treated with AAV-FIX-WT. At 14 days following injury, using microCT imaging and VBS analyses we observed a great improvement in pathological changes in the joints and bones of mice treated with FIX-Triple compared to those with FIX-WT. Mice receiving low viral vector doses to mimic those in low trough FIX levels will be tested.

Conclusion: We conclude that FIX-Triple is a potential therapeutic substitute for FIX-WT.

Disclosure of Interest: None declared.

PO030-MON

Standardizing enoxaparin dosing in mouse models of deep vein thrombosis

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Background: There are limitations to investigating venous thrombosis (VT) in humans. Thus, mouse models of VT are critical for studying mechanisms and testing new treatments. Enoxaparin (Lovenox®), an

anticoagulant used as the standard in VT research, serves as a control for the development of new antithrombotic drugs. However, there exists no published dosage data showing enoxaparin's effectiveness in mouse models of VT, supported by anti-Xa levels.

Aims: The objective of this work was to find an enoxaparin dose that produces anti-Xa levels in the therapeutic range ($0.5\text{--}1.0\text{ IU mL}^{-1}$) and significantly reduces thrombus weight (TW) in a flow and stasis VT model.

Methods: Male, 10 weeks old, C57BL/6 mice underwent either the Electrolytic Inferior Vena Cava (IVC) Model (EIM), or the IVC Ligation Model. Groups: Non-VT ($n = 9$), VT controls no treatment ($n = 10$) and VT enoxaparin groups ($n = 5$ per group) given in doses of 3, 4, 5, or 6 mg kg $^{-1}$, once daily, subcutaneously, post-surgery. Two days post-thrombosis blood was drawn for anti-Xa assays, and the IVC/thrombus weighed.

Results: The 4, 5 and 6 mg kg $^{-1}$ EIM enoxaparin groups had average therapeutic anti-Xa levels of: 0.56, 0.83 and 0.89 IU mL $^{-1}$, respectively, and in same ligation groups: 0.85, 0.81 and 0.83 IU mL $^{-1}$. In the EIM, there was a significant decrease in TW of 61–66% ($P < 0.05$) for 5 and 6 mg kg $^{-1}$. The 3 and 4 mg kg $^{-1}$ enoxaparin group did not yield significant TW reduction. The trend of the EIM experimental groups showed an inverse relationship between dosing and TW. In the ligation model, the therapeutic levels of anti-Xa levels were not reflected in the TW. The maximum decrease of 29%, vs. VT control group, was in enoxaparin 6 mg kg $^{-1}$ group.

Conclusion: These data reveal that for the EIM, 5 and 6 mg kg $^{-1}$ are the proper doses to significantly reduce TW and reach therapeutic anti-Xa levels. For the ligation model, 6 mg kg $^{-1}$ of enoxaparin is indicated. This work standardizes the enoxaparin dosing in these two mouse models of deep vein thrombosis.

Disclosure of Interest: None declared.

PO031-MON

Generation of hemophilia B mice in NSG background using CRISPR/Cas9 technology

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Background: Hemophilia B is caused by mutations in the factor IX gene. Approximately 3–5% of severe hemophilia B patients develop inhibitors after treatment with factor IX protein products. The inhibitors disable the function of infused FIX and patients are thereafter difficult to treat with the same factor IX products. The cause and risk factors for inhibitor development are not fully understood. A humanized mouse model will facilitate the investigation of mechanism(s) for the development of inhibitors.

Aims: The aim of this study was to establish a factor IX deficient mouse model in the NOD scid IL2rg $^{-/-}$ (Hemo B-NSG) genetic background.

Methods: CRISPR/Cas9 technology was used in this study. An oligonucleotide of 20 residues was designed for generating gRNA that targets the exon 1 of factor IX. The oligo was ligated to a T7 promoter sequence for *in-vitro* transcription to generate the gRNA. The CRISPR/Cas9 RNA was also *in-vitro* transcribed from a commercially available cDNA clone. The gRNA and the CRISPR/Cas 9 RNA was microinjected into the NSG mice zygote to generate founders.

Results: A total of 14 founders (6 male and 8 female) were analyzed for mutations in the factor IX gene. Of these, 3 contained mutations in

the factor IX gene. Two of the 3 were male and female containing the same mutation that had an 8 nucleotide deletion (from the 6th to 14th nucleotide after the A nucleotide of the 1st ATG) resulting in a premature stop codon at the 30th amino acid residue in exon 1. The other was a female mouse that contained a 2-nucleotide deletion at the 14th and 15th position that results in a premature stop codon at the 32nd amino acid residue in exon 1 of factor IX.

Conclusion: CRISPR/Cas9 technology is a very efficient method to generate hemophilia B disease model in a rare and difficult to breed mice. Our hemophilia B NSG mice will be a very good model for studying human immune response to factor IX products.

Disclosure of Interest: None declared.

PO032-MON

Acute bone loss in FVIII and FIX but not VWF deficient mice from joint injury

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Background: While joint damage is the primary co-morbidity of hemophilia, osteoporosis and increased fracture risk are also observed. We have previously shown that FVIII deficient mice develop an osteoporotic phenotype without hemarthrosis that is exacerbated 2 weeks after an induced joint injury. For this study, the work is expanded to include examination of FIX and vWF deficient mice. Reported here are bone changes as examined by microCT, but serum chemistry and histological analyses were also performed.

Aims: To examine the osteoporotic phenotype in three transgenic strains of mice with coagulation disorders and determine the relative contribution of reduced bone mass through development and induced joint injury.

Methods: Three transgenic strains of mice were used in this study, with each strain having corresponding littermate WT controls: FVIII $^{-/-}$, FIX $^{-/-}$, and vWF $^{-/-}$. Half of the mice from each group were subjected to a unilateral joint hemorrhage of the left knee at 22-weeks of age (INJ), while the other half were uninjured (UINJ). All mice were euthanized at 24-weeks of age. Hind limbs were processed for microCT; proximal tibia trabecular vBMD, and distal femur periosteal surface roughness ratio (SRR) are reported.

Results: Both UINJ FVIII $^{-/-}$ and UINJ FIX $^{-/-}$ groups had lower vBMD (–28% and –16%, respectively $P < 0.05$), but not the UINJ vWF $^{-/-}$ group, when compared to UINJ WT groups. INJ did not cause bone loss in any of the WT groups. For INJ FVIII $^{-/-}$ and INJ FIX $^{-/-}$ groups, but not INJ vWF $^{-/-}$, there was additional bone loss in the INJ limb compared to the UINJ limb (vBMD –30% and –24%, respectively). For SRR analysis, WT or UINJ groups were unaffected. INJ FVIII $^{-/-}$, INJ FIX $^{-/-}$ and INJ vWF $^{-/-}$ groups had significant changes in INJ limb SRR compared to UINJ limb (–7%, –8%, –1%, respectively).

Conclusion: FVIII $^{-/-}$ and FIX $^{-/-}$ mice, but not vWF $^{-/-}$ mice, developmentally have an osteoporotic phenotype. Unilateral induced hemarthrosis causes an acute loss of additional bone.

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PO033-MON

Enoxaparin acutely worsens hemodynamic measurements compared with tPA in a large animal model of submassive pulmonary embolism

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Background: Our previous published swine model of autologous Pulmonary Embolism (PE) has proven to be similar to submassive PE in humans. No model to date has compared treatments on the quantifiable hemodynamic parameters in an animal model of submassive PE with autologous clot.

Aims: Test traditional therapies of a swine model of PE with autologous clot.

Methods: *Sus scrofa* were anesthetized and intubated. Invasive monitoring were placed in the femoral artery for ABP measurements and Swan-Ganz catheter in the external jugular for pulmonary artery pressures (PAP), Cardiac Output (CO), and pulmonary vascular resistance (PVR). Autologous clots were delivered via a large bore catheter in the left external jugular until SBP < 90 mm Hg, PAP > 50 mm Hg or EtCO₂ < 40 torr. Treatment was either 1 mg kg⁻¹ of enoxaparin or 100 mg of tPA + enoxaparin. Measurements were recorded every 10 min. Clot was confirmed using echocardiography. Statistical tests used were t-test with a Bonferroni correction.

Results: A total of 23 animals underwent experimentation. Average clot burden was 20.21 g (SD ± 4.71) or 0.39% (SD ± 0.11). All animals showed similar increased in PVR (1617 ± 192 dyne·s cm⁻⁵), RV:LV ratio (1.23 ± 0.07), and PAP (53 ± 1/27 ± 1 mm Hg) after clot induction. Interestingly, enoxaparin alone appeared to worsen the hemodynamic measurements. Statistically significant heart rate (77 ± 21 vs. 66 ± 9) and oxygen saturation (93 ± 8 vs. 97 ± 3) were both higher with a subsequent lower etCO₂ (40 ± 8 vs. 46 ± 4) compared to no treatment in the enoxaparin group. PVR (1075 ± 186 vs. 1184 ± 133) and RV:LV ratio (1.13 ± 0.28 vs. 1.02 ± 0.02) remained comparable to no treatment with no statistical difference. In Contrast tPA showed improved PAP (45 ± 7/30 ± 5), O₂ sat (96 ± 4), PVR (813 ± 378), and RV:LV ratio (0.89 ± 0.04). No statistical difference was seen in either group compared to controls in regards to CO, or troponin.

Conclusion: Enoxaparin alone worsens while tPA + enoxaparin alleviate signs of RV strain in a controlled submassive swine model immediately after treatment.

Disclosure of Interest: None declared.

PO034-MON

Genome-wide knockdowns in zebrafish to study thrombocyte functionJagadeeswaran P, Sundaramoorthi H and Khandekar G
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Background: Thrombocytes are nucleated blood cells functionally equivalent to mammalian platelets which play a primary role in hemostasis. A substantial amount of research has been done to study hemostasis in humans as well as in large animal models and has resulted in the identification of more than two hundred hemostatic factors. Despite these achievements, given the complexity of the vertebrate genome, several hundred factors remain to be identified. We introduced zebrafish a vertebrate genetic model to study the genetics of hemostasis. However, the progress has been frustratingly slow due to the lack of suitable technology. Therefore, we invented a novel piggyback knockdown method that is both cost effective and germane to large-scale knockdowns. We also designed a quantitative thrombocyte functional assay by the use of flow cytometry. Thus, it should be feasible to

perform genome-wide knockdowns and identify genes that affect thrombocyte function.

Aims: The goal of this study is to perform genome-wide knockdowns and to comprehensively identify most genes involved in thrombocyte aggregation.

Methods: We used piggyback knockdown method and thrombocyte functional assay by flow cytometry in this study.

Results: Using this piggyback knockdown technology, and using thrombocyte functional assay by flow cytometry, we have conducted 3000 gene knockdowns and have identified over 75 genes involved in thrombocyte aggregation. Thus, by using the zebrafish model combined with the piggyback knockdown, it should be possible to establish a comprehensive list of genes involved in thrombocyte aggregation.

Conclusion: This work marks the beginning of genome-wide screens to study hemostasis by the rapid piggyback knockdown method.

Disclosure of Interest: None declared.

Antiphospholipid syndrome I

PO035-MON

Brain white matter microstructure is altered in obstetric antiphospholipid syndrome: evidences from diffusion-tensor MRI in asymptomatic neurological patientsGris J-CR^{1,2}, Lavigne G¹, Bouvier S¹, Nouvellon E¹, Mercier E¹, Pereira F³, Macri F³, Jackowski M³, Beregi J-P³ and Mekkaoui C³
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Radiology, University Hospital Caremeau, Nîmes, France: Background: Various brain pathologies are described in the thrombotic antiphospholipid syndrome (APS). The impact of antiphospholipid antibodies (aPLAbs) on brains of neurologically asymptomatic APS women with only obstetric manifestations is however controversial. Brain Diffusion Tensor MRI (DT-MRI) is a noninvasive method investigating neural connectivity by means of the water diffusion through tissues: this molecular 3D phenomenon reveals brain microstructural properties such as the anisotropy or the diffusivity in local regions.

Aims: To study brain DT-MRI aspects in obstetric APS women, by comparison to Control women sharing the same pregnancy loss antecedents. To analyse APS women after categorisation for aPLAb types.

Methods: We proposed brain DT-MRI to our regularly followed cohort of non-thrombotic women with previous pregnancy loss (NOH-APS), re-evaluated during a given month, with evidence of normal neuropsychiatric exams. Anisotropy level was accessed with fractional anisotropy (FA) index, diffusivity levels with mean and radial diffusivity (MD, RD) indices. Results in APS women were analysed according to the presence/absence of lupus-anticoagulant and/or anti-b₂-glycoprotein I IgG antibodies.

Results: 148 APS and Control women were proposed DT-MRI, 66 APS and 17 Controls women accepted this exam. APS patients presented white-matter (WM) reduction of anisotropy, associated to WM increase of diffusivity (MD and RD). The significance of these differences was more evident in APS women positive for lupus-anticoagulant (LA) and/or anti-b₂-glycoprotein I IgG antibody (ab₂GPI-G) than in APS women negative for both.

Conclusion: In non-thrombotic women sharing pregnancy loss antecedents with a normal neuropsychiatric evaluation, APS women showed DT-MRI aspects compatible with alteration of the axonal structure and of the myelin which culminated in individuals positive for LA and/or ab₂GPI-G. These infraclinic abnormalities may pave the way for future neuropsychiatric manifestations.

Disclosure of Interest: None declared.

PO036-MON

Thrombotic risk of reduced ADAMTS13 activity in patients with antiphospholipid antibodies

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Background: Antiphospholipid syndrome (APS) is an autoimmune disease in which antiphospholipid antibodies are generated. Previous studies show concurrence of APS and thrombotic thrombocytopenic purpura (TTP), therefore it is plausible to assume that anti-ADAMTS13 autoantibody is also involved in the pathophysiology of APS.

Aims: We investigated the clinical significance of ADAMTS13 activity and anti-ADAMTS13 antibody in patients with antiphospholipid antibodies.

Methods: Two hundred sixteen patients with positive lupus anticoagulant and/or anticardiolipin antibody and twenty normal controls were included in this study. ADAMTS13 activity and anti-ADAMTS13 antibody were measured using fluorescence resonance energy transfer technology and ELISA, respectively.

Results: Reduced ADAMTS13 activity was observed in 40.3% (87/216) of patients with antiphospholipid antibodies. Although 33.8% (73/216) of patients were positive for anti-ADAMTS13 antibody, 41 of these 73 patients had normal levels of ADAMTS13 activity. Reduced ADAMTS13 activity was a significant risk factor for thrombotic events. Thrombotic events and age were independently associated with the reduced level of ADAMTS13 activity. Presence of anti-ADAMTS13 antibody did not show any association with the level of ADAMTS13 activity. Patients with autoimmune diseases tended to show higher levels of anti-ADAMTS13 antibody.

Conclusion: Our findings suggest that reduced ADAMTS13 activity is a significant thrombotic risk factor in patients with antiphospholipid antibodies irrespective of the presence of anti-ADAMTS13 antibody. Presence of anti-ADAMTS13 antibody is not seen with reduced activity and it tends to be increased in patients with autoimmune diseases.

Disclosure of Interest: None declared.

PO037-MON

Mixing test specific cut-off is more sensitive at detecting *in vitro* lupus anticoagulant inhibition than the index of circulating anticoagulantMoore GW¹, Culhane AP¹, Daw CR¹, Noronha CP¹ and Kumano O²

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Background: Recent guidelines for lupus anticoagulant (LA) detection recommend assessing mixing tests for evidence of inhibition with either a mixing test-specific cut-off ratio (MTC) or the index of circulating anticoagulant (ICA). There are no studies directly comparing the diagnostic efficacy of both approaches.

Aims: To retrospectively apply MTC and ICA assessment to raw data of 350 LA-positive plasmas from non-anticoagulated patients to compare detection rates of inhibition.

Methods: Screen and confirm dilute Russell's viper venom time (dRVVT) and dilute activated partial thromboplastin time (dAPTT) assays were performed on undiluted plasma and 1:1 mixtures with normal pooled plasma on an automated, photo-optical clot detection coagulation analyser. Coagulation screening excluded other causes of elevated clotting times and undisclosed anticoagulation. Samples were considered LA-positive if one or both screening test ratios were above locally derived cut-offs and were corrected by $\geq 10\%$ with the confirmatory test. Mixing tests were assessed against locally derived cut-offs

for MTC (dRVVT > 1.13, dAPTT > 1.15) and ICA (dRVVT > 11.9%, dAPTT > 13.2%).

Results: One hundred five of 350 (30%) were positive in dRVVT and dAPTT, 109/350 (31.1%) were dRVVT positive only and 136/350 were dAPTT positive only (38.9%), from undiluted plasma results. Of the 214 dRVVT positive plasmas, 53 (24.8%) were negative for inhibition by MTC and 65 (30.4%) negative by ICA. Of the 241 dAPTT positive plasmas, 48 (19.2%) were negative for inhibition by MTC and 97 (40.2%) negative by ICA.

Conclusion: Whilst integrated testing often reveals the presence of LAs without mixing tests they are useful in certain circumstances and improve diagnostic specificity. Thus, it is valuable to maximise the diagnostic potential of mixing tests in LA detection as the dilution effect can lead to false-negative results. These data on a large cohort of LA-positive plasmas reveal that MTC is superior to ICA in detecting the *in vitro* inhibition of LA.

Disclosure of Interest: G. Moore: None Declared, A. Culhane: None Declared, C. Daw: None Declared, C. Noronha: None Declared, O. Kumano Employee of: Sysmex Corporation.

PO038-MON

A multicontrol kit for antiphospholipid and anti-platelet factor 4-heparin antibodies: nine different analytes in a single vialPuig J, Cavalcanti F, Torres R, Matilla S, Serra J and Marti T
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Background: At present, the quality control of antiphospholipid (aPL) and anti-Platelet Factor 4 (PF4)-heparin antibodies measured with the HemosIL[®] AcuStar assay panel is carried out by assessing two controls (low and high) for each assay. It is necessary to use several control vials to confirm the quality of the assay panel. Including all of these antibodies in the same preparation permits the use of a single vial for each level of Quality Control thereby minimizing the number of materials required.

Aims: To check the performance of the HemosIL AcuStar Multi-Ab Controls (Instrumentation Laboratory, Bedford, MA, USA) kit in terms of precision, real-time stability, on-board and daily/weekly use stability, when used for the Quality Control of nine assays: anti-B2GPI IgM, anti-cardiolipin (aCL) IgM, anti-B2GPI IgG, anti-B2GPI Domain 1, aCL IgG, anti-B2GPI IgA, aCL IgA, HIT-Ab and HIT-IgG.

Methods: AcuStar Multi-Ab Controls are prepared by means of a dedicated process and contain different concentrations of human antiphospholipid antibodies (IgG, IgM, and IgA) and a humanized PF4-Heparin monoclonal antibody. The performance has been assessed following Clinical and Laboratory Standards Institute protocols.

Results: Total precision (% CV) of the different analytes has been between 2.5–7.3% (low level) and 2.1–6.3% (high level). Real-time stability at 2–8 °C of the Control kit is 12 months. On-board stability of the open vial continuously on-board of the ACL AcuStar analyzer is 10 h. Daily usage (1 session of 45 min per day), 11 uses over 14 days and the weekly usage (1 session of 45 min per week), 11 uses over 10 weeks.

Conclusion: HemosIL AcuStar Multi-Ab Controls can be used for the Quality Control of seven aPL assays and two anti-PF4-Heparin assays on the ACL AcuStar analyzer with an equivalent performance to the individual Quality Control materials.

Disclosure of Interest: J. Puig Employee of: Biokit R&D, F. Cavalcanti Employee of: Biokit R&D, R. Torres Employee of: Biokit R&D, S. Matilla Employee of: Biokit R&D, J. Serra Employee of: Biokit R&D.

PO039-MON

Persistent lupus anticoagulant in clinical settings other than antiphospholipid syndrome

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Background: Lupus anticoagulant (LA) can be present in asymptomatic subjects or patients with clinical symptoms other than antiphospholipid syndrome (APS).

Aims: In order to assess the presence and persistence of LA in subjects with or without clinical criteria for APS (APSc), a 3 year retrospective analysis (2014–2011) was done. Patients with APSc and subjects with other pathologies or LA+ tests were included. All LA+ were re-evaluated (after > 12 weeks).

Methods: Subjects: 263 individuals were included; 211 (APSc) had at least one APSc, 52 (NO-APSc) were tested because they had pathologies possibly associated with LA (33) or LA+ results (19). Laboratory: routine tests (PT,TT), sensitive APTT and dRVVT were done; mixing/confirmatory tests were carried out on prolonged tests.

Results: LA+ (SSC-ISTH criteria) was detected in 185 subjects (142 APSc and 43 NO-APSc) (1st study). Among NO-APSc, LA+ was seen in bleeders (10/43), asymptomatic subjects (family history of bleeding or thrombosis) (9/43) and other pathologies (24/43) (infertility:15; chronic renal failure on haemodialysis:7; coronary arterial disease:2).

The 2nd study showed persistent LA in 100/211 APSc (47.4%) and 33/52 NO-APSc (63.5%), including 8/10 bleeders, 6/9 asymptomatic and 19/24 subjects with 'other' manifestation (infertility: 5 had transient LA). Interestingly 8/33 (24.2%) were bleeders; 5/8 had other abnormal tests that maybe would explain their symptoms (platelets dysfunction:2; hyperfibrinolysis:1; low FXI:1; low Fg + low platelet count:1).

Among APSc, 42/142 became LA- in the 2nd study; 19/42 were considered transient LA (LA- in a 3rd study) and 23/42 require a 3rd evaluation in order to define persistence.

Conclusion: Persistent LA is a frequent finding in asymptomatic subjects and in patients with bleeding history. A careful analysis of laboratory results should be done, even if the diagnosis of an haemorrhagic disorder is not always achieved.

Disclosure of Interest: None declared.

PO040-MON

How appropriate is the antiphospholipid antibodies testing at Siriraj Hospital?

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Background: Diagnosis(Dx) of antiphospholipid syndrome(APS) requires ≥ 1 clinical event (thrombosis/pregnancy morbidity) and ≥ 1 positive antiphospholipid antibodies(aPL) test on ≥ 2 times ≥ 12 weeks apart.

Aims: To evaluate how appropriate aPL tests were sent and identify factors associated with the Dx of APS and positive aPL test

Methods: Retrospective chart review of 402 patients (292 women) with aPL tests during Feb 2011 and Jan 2012 was done. The appropriateness of sending aPL tests according to the aPL Subcommittee of the ISTH guidelines 2009, was categorized into three levels (low, medium and high). Patients with clinical criteria with 1 positive aPL test on 1 occasion only was defined as possible APS.

Results: The median age was 41 years (range 18–90). Clinical events included thrombosis (58.4%), which comprised venous thromboembolism(VTE;36.6%) and/or arterial thrombosis(AT; 25.9%) and pregnancy morbidity (11.5%). The coexisting diseases included SLE (28.9%), other autoimmune(27.9%) and malignancy(8.2%). Indication for aPL testing included Dx of APS(78%), Dx of SLE(15.9%) and others(6%). Dx of APS was made in 66(16.4%) patients (31 definite and 35 possible APS). For the indication for Dx of APS, the level of appropriateness of testing was mostly in high level(50%) while 33.1% and 16.9% was in low and medium levels. Compared to the low level of appropriateness of testing, the high level significantly associated with definite APS (OR 11.5; 95% CI 2.6–49.6). In multivariate analysis, AIHA (OR 11.8; 3.5–39.5) and thrombosis at age ≤ 45 years (OR 3.3; 1.2–8.6) were associated with definite APS. In multivariate analysis, factors significantly associated with a positive aPL test were hemoglobin < 10 g dL⁻¹ (OR 5.6; 1.5–20.5) and ITP (OR 7.0; 1.0–47.6) whereas explained AT was negatively associated (OR 0.12; 0.03–0.43).

Conclusion: aPL testing at Siriraj hospital was mostly in high to medium appropriateness levels. AIHA and thrombosis at age ≤ 45 years correlated with definite APS. Anemia and ITP associated with positive aPL test.

Disclosure of Interest: None declared.

PO041-MON

Thrombomodulin down-regulation is related to the thrombosis risk in antiphospholipid syndrome patients

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Background: Antiphospholipid antibody (aPL) is associated with an increased risk of thrombosis. Although tissue factor (TF) up-regulation in cultured endothelial cells and monocytes was reported, the thrombosis mechanism remains unknown.

Aims: We focused on thrombomodulin (TM) to suppress thrombosis and investigated whether the expression was affected by aPL.

Methods: IgG in normal and APS patient were purified by Protein G column. Human umbilical vein endothelial cells were incubated in medium (–) IgG, 0.2 mg mL⁻¹ normal IgG, 0.2 mg mL⁻¹ aPL IgG, respectively. Medium includes 5 mg mL⁻¹ b2GPI, 10 ng mL⁻¹ LPS and 2% FBS, and HUVEC were incubated with each IgG for 4 h. mRNA levels of TF and TM were measured by RT-PCR. In addition, 5 mM cAMP was added to the medium to investigate the effect. The mRNA level in (–) IgG was defined as 100%.

Results: TF mRNA levels in (–) IgG, normal IgG and aPL IgG were 100%, 120% and 215%, respectively. In this situation, TM mRNA levels were 100%, 72% and 43%, respectively. For 5 mM cAMP, TF mRNA levels were 68%, 66% and 61%, respectively, and TM mRNA levels were 161%, 112% and 74%, respectively.

Conclusion: TM mRNA level was decreased in medium with aPL, and cAMP enhanced this effect. The decreasing of TM to suppress coagulation is related to thrombosis, and it was considered that one of the thrombosis mechanism of APS was TM expression decreasing. Furthermore, TF expression was not increased in high cAMP concentration, while decreasing of TM expression was recognized. cAMP was stimulated by several signals such as shear stress, and also relates to the thrombosis risk. Thrombosis in Antiphospholipid syndrome patients could be derived from not only TF up-regulation but also TM down-regulation.

Disclosure of Interest: O. Kumano Employee of: Sysmex, M. Ieko Grant/Research Support from: Sysmex, S. Naito: None Declared, M. Yoshida: None Declared, N. Takahashi: None Declared, T. Suzuki Employee of: Sysmex, T. Aoki: None declared.

PO042-MON

Use of D Dimer in biological control of pregnancyOtero AM, Lena AM, Lisa ED, Attarian D, Pons E, Pou R, Mota N, Domínguez G, Santos SDL and Carrizo C

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Background: Use of low molecular weight heparin (LMWH) and low-dose aspirin has been accepted as the best treatment for pregnant women with antiphospholipid syndrome since many years ago. In spite of this treatment being very effective, many patients present complications. Anti-Xa only gives information about the level of heparinemia.

Aims: Demonstrate that D-Dimer ELISA, as a marker of coagulation activation, would be useful to detect pregnancy complications and decide the increase of LMWH dose if necessary, in pregnant women with antiphospholipid syndrome.

Methods: A total of 220 pregnant women with antiphospholipid syndrome treated with LMWH.

D-dimer was determined monthly by ELISA methodology (Asserachrom D-DI (Diagnostica Stago, France).

It was considered an abrupt increase of DDimer, when the title increased at least twice, comparing results from one month to the next month.

We calculated the sensitivity, specificity, positive and negative predictive value of D-dimer ELISA to detect pregnancy complications in pregnant women with antiphospholipid syndrome, treated with LMWH.

Complications during pregnancy were confirmed by eco Doppler.

Results: The fifth part of women included in this study presented an abrupt increase in D dimers, 88% of them presented pregnancy complications, 6% presented urinary infection and there were not causes that explained the abrupt increase in 6%. Considering the remaining 4/5 parts of the patients, only 2% presented pregnancy complications, in spite of not having presented an abrupt increase of D dimers.

Conclusion: D-dimer ELISA has a high sensitivity and specificity for detecting complications during pregnancy in patients with antiphospholipid syndrome treated with LMWH, with a high positive and negative predictive value. Its use also allows to increase the dose of LMWH if necessary to improve the effectiveness of the treatment.

Disclosure of Interest: None declared.

PO043-MON

Anti-annexin V antibodies in patients with obstetric morbidityManeyro A, Ouviaña S, Noya L and Palmer L

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Background: Annexin-V (AnxV) is an anionic phospholipid-binding protein with potent anticoagulant activity. It inhibits prothrombin activation and can prevent thrombus formation under normal blood flow conditions. It has been suggested that displacement of the AnxV shield from the syncytiotrophoblast surface by anti-Annexin V antibodies (ab-AnxV) creates a thrombogenic environment responsible for the subsequent fetal loss. So far, the laboratory criteria for the diagnosis of the Antiphospholipid Syndrome (APS) includes Lupus Anticoagulant, anti-Cardiolipin antibodies and anti-beta2-glycoprotein I determinations.

Aims: To evaluate if testing for ab-AnxV may help to the diagnosis in patients with pregnancy morbidity associated with APS.

Methods: We evaluated 117 patients (p) seen in our institution, for the presence of antiphospholipid antibodies, following ISTH laboratory criteria. Patients were divided in 2 groups: Group 1 (30p) with pregnancy complications suggestive of APS and Group 2 (87p) with venous thromboembolic disease, collagenopathies and cardiovascular events. All patients underwent Ab-AnxV tested by ELISA method.

Results: In Group 1 Ab-AnxV positivity was found in 6 p (20%); in 2 p (6.7%) Ab-AnxV was the only positive antiphospholipid antibody found.

In Group 2 Ab-AnxV positivity was found in 11p (12.6%); in 4 p (4.6%) Ab-AnxV was the only positive antiphospholipid antibody found.

Conclusion: In spite of the small sample size, we think that Ab-AnxV evaluation may be a useful diagnostic tool in p with clinically suspected APS, adding to the classical laboratory determinations, particularly in those women with pregnancy complications, and may identify a subgroup of patients with a clinical picture highly suggestive of APS but negative laboratory criteria. If these findings are confirmed in large scale studies, we think that Ab-AnxV should be added to the laboratory criteria of APS.

Disclosure of Interest: None declared.

PO044-MON

Interactions between rivaroxaban and antiphospholipid antibodies in thrombotic antiphospholipid syndromeArachchillage DJ¹, Mackie I¹, Efthymiou M¹, Isenberg D², Machin S¹ and Cohen H^{1,3}¹Haemostasis Research Unit, University College London; ²Centre for Rheumatology, Division of Medicine, University College London; ³University College London Hospitals NHS Foundation Trust, London, UK

Background: Rivaroxaban can affect lupus anticoagulant (LA) tests, and antiphospholipid antibodies (aPL) may interfere with rivaroxaban anticoagulant action.

Aims: To establish the influence of rivaroxaban on LA detection and of aPL on rivaroxaban anticoagulant action.

Methods: Rivaroxaban and 52 IgG preparations (20 LA positive, 12 LA negative thrombotic antiphospholipid syndrome [APS] patients, 20 normal controls [NC]), were spiked into pooled normal plasma (PNP) for LA testing. aPL effects on rivaroxaban anticoagulant action were studied by thrombin generation (TG) using a calibrated automated thrombography and rivaroxaban anti-Xa assay. LA was also studied in thrombotic APS patients receiving rivaroxaban 20 mg once daily, at peak and trough levels.

Results: *In vitro* spiking of samples with rivaroxaban showed no false positive LA with Textarin time, Taipan venom time/Ecarin clotting time (TVT/ECT), dilute prothrombin time (dPT) and in-house dilute Russell's viper venom time (DRVVT); but false positives in the majority of NC and LA negative IgG (18/20 [90%] and 11/12 [92%] respectively) with two commercial DRVVT reagents, at 250 ng mL⁻¹ but not 50 ng mL⁻¹ rivaroxaban (expected peak and trough levels respectively). *Ex vivo* studies: six LA positive patients on rivaroxaban remained LA positive with TVT/ECT and both DRVVT reagents, at peak (162–278 ng mL⁻¹) and trough (30–85 ng mL⁻¹) rivaroxaban levels. Six LA negative patients became (apparently) LA positive with both DRVVT reagents, test/confirm ratio median [CI]: 1.6 [1.3–1.8], 1.6 [1.4–1.9], but not by TVT/ECT (1.1 [0.8–1.2]) at peak rivaroxaban levels; and remained LA negative with both DRVVT reagents and TVT/ECT at trough levels. aPL positive IgG spiking of PNP had no effect on rivaroxaban anticoagulant effects on thrombin generation or rivaroxaban anti-Xa levels.

Conclusion: The TVT/ECT ratio and Textarin time were not affected even at peak rivaroxaban levels, enabling detection of LA *ex vivo*. aPL had no effects on rivaroxaban anticoagulant action *in vitro*.

Disclosure of Interest: None declared.

PO045-MON

Proposed trial: hypatia – a prospective randomised controlled trial of hydroxychloroquine versus placebo during pregnancy in women with antiphospholipid antibodies

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Background: Antiphospholipid syndrome (APS) is defined by the presence of antiphospholipid antibodies (aPL) with thrombosis and/or obstetric morbidity. The obstetric morbidity includes recurrent first trimester loss, stillbirth, intrauterine death, pre-eclampsia, premature birth and fetal growth restriction.

Current treatment including low dose aspirin and low molecular weight heparin have improved pregnancy outcome, but reduction in pregnancy loss remains sub-optimal.

The antimalarial hydroxychloroquine (HCQ) has been used for decades in patients with SLE and our retrospective single centre study suggests that HCQ has a favourable effect on pregnancy outcomes and thrombosis. HCQ is recognised to be safe in pregnancy. Therefore an RCT of HCQ vs. placebo in women with antiphospholipid antibodies is proposed.

Aims: To undertake a randomised controlled trial of HCQ vs. placebo in addition to standard treatment in women with aPL in order to improve pregnancy outcome.

Methods: We propose the first multicentre RCT of HCQ vs. placebo in addition to standard treatment in pregnant women with aPL or APS. A total of 328 women will be enrolled.

Results: The primary endpoints will include: miscarriage < 10 weeks of gestation, miscarriage > 10 weeks, premature birth < 34 weeks due to eclampsia, pre-eclampsia and/or intrauterine growth restriction and/or placental abruption. Secondary endpoints will include gestational age at delivery, birth weight, mode of delivery, APGAR-score, neonatal morbidity, bilateral uterine artery notching between 20 and 24 weeks, days to discharge from hospital following delivery (mother and baby) and thrombotic events in the mother and the baby.

Conclusion: The proposed trial will address the issue of the utility of hydroxychloroquine in improving pregnancy outcome in women with aPL. We invite other centres to express interest in joining us.

Disclosure of Interest: None declared.

PO046-MON

Rivaroxaban limits complement activation in patients with thrombotic antiphospholipid syndrome

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Background: Complement activation has been shown to play a role in the pathogenesis of thrombosis in antiphospholipid syndrome

(APS). We hypothesize that rivaroxaban, as a direct factor Xa inhibitor, could limit complement activation.

Aims: To assess the classical (C3a and C5a) and terminal complement complex, SC5b-9, in patients with thrombotic APS treated with rivaroxaban compared to warfarin in a prospective randomised study.

Methods: Sixty APS patients with previous venous thromboembolism, without systemic lupus erythematosus, on long term warfarin target INR 2.5 (2.0–3.0), were studied: 30 remained on warfarin and 30 switched from warfarin to rivaroxaban (20 mg daily). EDTA plasma samples were collected at baseline (all on warfarin) and day 42 (30 on warfarin, 30 on rivaroxaban). Samples were also collected from 30 normal controls (NC). C3a, C5a and SC5b-9 were assessed using ELISA assay kits (QUIDEL Corp).

Results: Normal ranges for C3a, C5a and SC5b-9 were 42.3–57.1 ng mL⁻¹, 4.9–7.8 ng mL⁻¹, 106–128 ng mL⁻¹ respectively. APS patients had significantly higher complement activation markers compared to NC at both time points irrespective of the anticoagulant ($P < 0.0001$ for C3a, C5a and SC5b-9). There was no difference in these markers between the two patient groups (all on warfarin) at baseline. On day 42, in patients remaining on warfarin, median C3a, C5a and SC5b-9 were similar to baseline: C3a (ng mL⁻¹) 81.4 vs. 88.5, C5a (ng mL⁻¹) 11.2 vs. 11.7, SC5b-9 (ng mL⁻¹) 212 vs. 205. In patients treated with rivaroxaban (day 42), median C3a, C5a and SC5b-9 were significantly lower compared to warfarin (baseline): C3a (ng mL⁻¹) 74.3 vs. 89.2 ($P = 0.04$), C5a (ng mL⁻¹) 8.2 vs. 10.3 ($P = 0.04$), SC5b-9 (ng mL⁻¹) 162 vs. 197 ($P = 0.02$). However, there was no correlation between rivaroxaban anti-Xa levels and C3a, C5a and SC5b-9.

Conclusion: Rivaroxaban appears to inhibit activation of the complement pathway compared to warfarin. These data suggest that rivaroxaban has additional therapeutic modality in thrombotic APS patients by limiting complement activation.

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PO048-MON

Analytical and clinical performance of a new automated chemiluminescent assay (CIA)

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Background: Laboratory classification criteria of antiphospholipid syndrome (APS) include the presence of lupus anticoagulant together with anticardiolipin (aCL) and antiβ₂-glycoprotein I (aβ₂GPI) IgG and IgM antibodies, detected by enzyme-linked immunosorbent assays (ELISA) containing human β₂-glycoprotein I on a solid phase.

Aims: To assess the analytical performance and clinical utility of a fully automated anticardiolipin (aCL) and anti-β₂ glycoprotein I (aβ₂GPI) chemiluminescent immunoassays (CIA) and their profiles in comparison to the traditional ELISA tests.

Methods: The study included 220 samples from patients with primary APS ($n = 74$), secondary APS ($n = 47$), and control sera from patients with systemic lupus erythematosus (SLE) without APS ($n = 99$). All samples were tested for IgG and IgM aCL and β₂GPI antibodies using the ELISA methods (QUANTA Lite aCL, aβ₂GPI ELISA; Inova Diagnostics, USA) and the CIA methods (QUANTA Flash aCL, aβ₂GPI; Inova Diagnostics, USA). Data were statistically evaluated using Analyse-it for Microsoft Excel 3.9 (Analyse-It Software, Leeds, UK).

Results: Good qualitative agreement was found between CIA and ELISA methods, with overall agreements ranging from 81.8% (aCL IgM assays) to 90.5% (aCL IgG assays). All assays showed good clinical performance, and strong correlation with APS-related clinical symptoms. For aCL IgG, sensitivity and specificity were comparable. For the IgM class, sensitivity of the ELISA test was higher (70.2%) than of the CIA method (33.9%), but the specificity was higher for the CIA (91.9% vs. 72.7% for ELISA). For a β 2GPI IgG antibodies, the sensitivity was lower for the ELISA test compared to the CIA (55.4% vs. 71.1%) but the specificity was higher for ELISA (87.9% vs. 76.8%). For the IgM class of a β 2GPI, sensitivity and specificity were similar for both methods.

Conclusion: This study showed good clinical performance and strong correlation of the new automated CIA aPL assays with the APS clinical symptoms.

Disclosure of Interest: None declared.

PO049-MON

False positive results of platelet neutralization procedure (PNP) in lupus anticoagulant (LA) testing of patients with coagulation factor deficiencies

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Background: Laboratory testing for LAs utilizes a phospholipid (PL)-rich confirmatory phase reagent. ISTH 2009 guidelines state, 'Freeze/thawed platelets are not recommended as the source of PL for the confirmatory tests because of poor batch-to-batch consistency'. We explored potential additional limitations of such reagent.

Aims: We investigated PNP assay specificity.

Methods: Instruments and reagents: STA-R Evolution/PTT-LA, Cryocheck Platelet Lysate (Precision BioLogic).

Results: Patient 1 presented with an AV fistula thrombosis, prolonged aPTT (104"), and normal PT. PTT-LA screen was markedly prolonged (120.3", normal < 44") with a normal thrombin time. PTT-LA mix was marginally prolonged (37.4", normal < 37.2"). In the confirmatory PNP, there was dramatic shortening (8.3 SD above the mean, normal < 3.0 SD). DRVVT was negative for a LA. Subsequent analyses revealed normal or high levels of factors (F) VIII, IX, XI, XII and high molecular weight kininogen, while prekallikrein (PK) was severely deficient (< 1%).

Patient 2 had a post-operative consumptive coagulopathy with prolonged PT (27.6") and aPTT (43.9"), multiple factor deficiencies (FII 27%, FV 57%, FVII 19%, FX 30%, FXI 39%), decreased platelets (111K), and D-Dimer > 20 μ g mL⁻¹. PTT-LA screen was prolonged at 76.7" with only partial correction in the mixing phase (47.6"), and highly abnormal PNP confirmatory phase (21.3 SD). DRVVT and DPT testing was normal.

Utilizing FXI-deficient reagent substrate plasma in the PNP produced an entirely normal result. In contrast, study of FV-deficient reagent substrate plasma in the PNP produced a markedly abnormal shortening of clotting time in the presence of platelet lysate, exceeding 77 SD above the mean.

Conclusion: Freeze-thawed platelet lysate reagents offer a relatively physiologic PL source. However, such platelet lysates may not only show lot-to-lot variation, but also produce false positive LA testing results in patients with deficiencies of platelet-associated coagulation factors, such as PK and FV.

Disclosure of Interest: None declared.

PO050-MON

Frequency of clinically significant antiphospholipid antibodies profile in patients with venous thromboembolism, stroke, myocardial infarction, peripheral arterial disease and pregnancy morbidity

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Background: antiphospholipid antibodies (aPL) frequency in patients with thrombosis or pregnancy morbidity is uncertain for the lack of standardized laboratory diagnostic criteria. The estimated aPL frequency is 6% for pregnancy morbidity (PM), 13.5% for stroke, 11% for myocardial infarction (MI), and 9.5% for deep vein thrombosis (DVT) (Arthritis Care & Research; 2013, 1869).

Aims: to evaluate the rate of clinically relevant aPL profile according to Sydney international consensus (2006) in patients with arterial or venous thromboembolism or PM.

Methods: patients referred to our specialized coagulation laboratory from 2004 to 2014 with prior DVT, pulmonary embolism (PE), superficial vein thrombosis (SVT), stroke, transient ischemic attacks (TIA), MI, peripheral arterial disease (PAD) and PM underwent testing for lupus anticoagulant (LAC) according to criteria of the Subcommittee for LAC/aPL of ISTH of 1995 updated in 2009, anti-cardiolipin antibodies (ACA) IgG/IgM and anti-beta2glycoprotein I (GPI) IgG/IgM, according to the International Consensus Guidelines on ACA and anti-beta2 GPI (Arthritis and Rheumatism, 2012).

Results: Table 1 shows the study results in the different populations. Patients with isolated LAC were < 1% in all subgroups of patients, while patients with LAC and one or both ELISA tests positive for either ACA and/or anti-beta GPI-2 were similarly low except for patients with proximal DVT (1.4%).

	No. pats	No. with (%) LAC (negative ELISAs)	No. (%) with LAC + positive ELISAs	No. (%) with LAC
Patients				
Prox DVT	4637	34 (0.7)	64 (1.4)	98 (2.1)
Distal DVT	975	7 (0.7)	7 (0.7)	14 (1.4)
PE	819	2 (0.2)	3 (0.4)	5 (0.6)
SVT	2247	21 (0.9)	7 (0.5)	28 (1.2)
Stroke	1746	14 (0.8)	12 (0.7)	26 (1.5)
TIA	1566	11 (0.7)	11 (0.7)	22 (1.4)
PAOD	1101	11 (1.0)	10 (0.9)	21 (1.9)
Obstetrical complications	1371	7 (0.5)	4 (0.9)	11 (0.8)

Conclusion: A clinically significant aPL profile diagnosed according to updated laboratory criteria has a much lower frequency than that reported in subjects with thrombosis and pregnancy complications.

Disclosure of Interest: None declared.

PO051-MON

Clinical assessment of antiphospholipid antibodies against β 2-glycoprotein domain I in antiphospholipid syndrome

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Background: The antiphospholipid syndrome (APS) is characterized by thrombosis and positivity of at least one of antiphospholipid anti-

bodies (APL): anticardiolipin (ACL), anti β 2 glycoprotein I (anti- β 2GPI) and lupus anticoagulant. The assessment of biological markers of thrombosis is challenging in APS. In fact, a significative number of patients with APL remain asymptomatic. Antibodies against β 2glycoprotein IgG domain I (anti β 2GPIgG domain I) are prevalent in APS. However, they are not performed in the routine laboratory and are not included in the Sydney criteria.

Aims: To assess the clinical impact of anti β 2GPIgG domain I in APS:

Methods: Eighty-two positive-anti β 2GP IgG patients were included. 12% were asymptomatic ($n = 10$), 22% had autoimmune disease ($n = 18$), and 66% with APS ($n = 54$). APL were carried out in a new-fully automated chemoluminescent analyser (ACL AcuStar IL). We used the SPSS V20 program for statistical analysis and $P < 0.05$ had significant value.

Results: Anti β 2GPIgG domain I were positive in 70% of APS, 28% of autoimmune diseases and in 30% of asymptomatic patients. The sensitivity for thrombosis was 71% and the specificity was 70%. The positive predictive value (PPV) was 55% and the negative predictive value (NPV) was 83%. ACLIgG were positive in 78% of APS, 55% of autoimmune diseases and 40% of asymptomatic patients. ACLIgG sensitivity was 78% and the specificity was 50%. The PPV was 75% and the NPV was 54%. Forty patients had both anti β 2GPIgG domain I and ACLIgG positivity, 82% of them with APS.

Conclusion: Anti β 2GPIgG domain I are more prevalent in patients with thrombosis. The high NPV could identify patients at low risk for thrombosis. Therefore, a negative anti β 2GPIgG domain I would be considered a low thrombotic risk marker. Moreover, patients with triple positivity (ACLIgG, anti β 2GPIgG domain I and anti β 2GPI) could be considered at high risk of APS. However, multicentric studies are needed to obtain clinical relevant results.

Disclosure of Interest: None declared.

PO052-MON

Multiple positivity and APA profile in women with fetal loss syndrome

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Background: As it is known pregnancy loss is a marker and the most striking manifestation of APS. We wanted to evaluate the importance of different antiphospholipid antibodies, especially antibodies to annexin V and anti-prothrombin antibodies that are not included in diagnostic criteria of APS.

Aims: The evaluation of APA profile in patients with fetal loss syndrome.

Methods: We have investigated anticardiolipin, antiannexin V, anti- β 2-GPI, antiprothrombin antibodies using ELISA method and LA circulation in 146 women with history of recurrent miscarriage and 60 age matched healthy pregnant women. The study included 74 first trimester pregnant women (I group) and 72 s and third trimesters women (II group) who had a history of unexplained recurrent miscarriage.

Results: About 34.2% women were diagnosed APS. LA circulation – 14%, anticardiolipin – 31.5%, anti-annexin V – 31%, anti- β 2-GPI – 22.6, anti-prothrombin – 10.3%. Combination of LA, anti- β 2-GPI, anticardiolipin was in 12.1%, LA, anti-annexin V and anti- β 2-GPI – in 13.7%, anti-prothrombin and anti- β 2-GPI – in 8.9%, LA, annexin V and β 2-GPI – in 7.9% and was associated with more severe complications. All APS patients received anticoagulant therapy. In women treated before the pregnancy early miscarriage in the next pregnancy occurred in 1.6%. There was no antenatal death or stillbirth. In II group the frequency of obstetric complications was higher compared with women of I group ($P < 0.05$) but still significantly lower compared their history without therapy.

Conclusion: In spite of the fact that antibodies to annexin V are not included to the APS criteria, their level prevailed in the women with history of fetal loss. We consider it as a risk factor of recurrent fetal

loss. Also the combination of various antibodies at the same time in women with more severe obstetric history demonstrates the diagnostic value of the determination of different groups of APA.

Disclosure of Interest: None declared.

Antiplatelets agents I

PO054-MON

Impact of preanalytical delay on results of light transmittance aggregometry in stemi patients

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Background: One explanation for the paradoxal laboratory response (PR) to clopidogrel, in which the medication leads to an increase in platelet aggregation (PA) compared with the initial value, is the pre-analytical delay of assessment after blood withdrawal.

Aims: Whether delay in assessment of PA by light transmission aggregometry (LTA) after blood sampling in STEMI patients results in a reduction rate of PA being false, which is regarded as PR to clopidogrel in the course of repeated examination.

Methods: Venous blood samples for the study of PA by LTA were withdrawn from 6 pts with STEMI, who were admitted for urgent PCI, prior to the intake of a loading dose of clopidogrel (600 mg). Samples were stored at ambient condition, and PA was evaluated at 1, 4, 8 and 12 h. Repeated blood samples for PA were collected and tested by the same method after 48 ± 6 h after PCI. PR to clopidogrel were recorded at Resistance Index (RI) < 0 . The control group included six volunteers of the same sex and age with no relevant medical history/concomitant medication.

Results: For STEMI pts, PA was significantly less than in control group after 1, 4 and 8 h, $P = 0.02; 0.03$ and 0.02 . 48 ± 6 h after PCI the PA was 28.8% (11.2; 41.7), that is significantly < 1 h after the initial blood sample ($P = 0.04$) and is not different from the PA determined in the blood at 4, 8 and 12 h. Cross differences were significant in determining the RI at 1, 4, 8 and 12 h: 54% (24.6; 84.3); 42.9% (8.6; 79.9); 62.9% (–52.2, 71.1); 37.1 (–60.9; 56.8), ANOVA $P = 0.016$. PR to clopidogrel was registered for 1 of 6 pts when evaluating at 1 and 4 h after blood sample collection (17%); and for 2 of 5 pts at 8 h (40%).

Conclusion: PA has decreased during storage of blood for more than 1 h, which can lead to an erroneous assessment of clopidogrel efficacy. False PR is registered with samples for LTA deferred over 8 h. Correct evaluation may be accomplished using personalized devices to assess PA. This study was supported by the project RFMEFI60714X0025.

Disclosure of Interest: None declared.

PO055-MON

Association of platelet response to cilostazol with clinical outcome and CYP genotypes in patients with cerebral infarction

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Background: Recent clinical trials have demonstrated that cilostazol, an inhibitor of phosphodiesterase 3, is a more effective and safer alternative

to aspirin for secondary prevention of cerebral infarction. Although the platelet responses to aspirin and clopidogrel have been reported to be associated with cardiovascular outcome, it is unclear whether the platelet response to cilostazol has the similar clinical implication.

Aims: To assess whether low response to cilostazol is associated with recurrence of cerebral infarction and with CYP genotypes.

Methods: We conducted a prospective observational study in 162 patients with cerebral infarction taking cilostazol. Cilostazol-induced phosphorylation of vasodilator-stimulated phosphoprotein (VASP) in platelets was measured by a recently developed assay which was modified from a platelet VASP assay kit that has been widely used for monitoring the platelet response to clopidogrel (Platelets 22: 135–142, 2011). CYP genotypes were determined by direct sequencing of the CYP2C19 and CYP3A5 genes that were involved in the metabolism of cilostazol. Recurrence of cerebral infarction during the follow-up period was determined by clinical symptoms and brain MRI imaging. This study was approved by Ehime University ethics committee.

Results: The median duration of follow-up was 24 months. The recurrence of cerebral infarction occurred in 6 (4.1%) of 146 patients who could define the clinical outcome. The patient group with recurrence had a lower level of VASP phosphorylation than the patient group without recurrence ($P = 0.045$). The genotypes of CYP2C19 and CYP3A5 were not associated with the level of VASP phosphorylation in 162 patients taking cilostazol.

Conclusion: This study suggests that low response to cilostazol detected by VASP assay is associated with the recurrence of cerebral infarction. The VASP assay for cilostazol may be useful to predict the clinical outcome in patients with cerebral infarction.

Disclosure of Interest: None declared.

PO056-MON

Platelet reactivity based risk assessment for patients with coronary artery disease on P2Y₁₂ inhibitors is not consistent between different platelet function tests

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Background: Patients with coronary artery disease (CAD) requiring P2Y₁₂ inhibitors are at risk of bleeding as well as recurrent ischemia. On-treatment platelet reactivity (PR) has been related to the risk of these events, and for several platelet function tests (PFTs) therapeutic windows of PR have been proposed.

Aims: To determine the consistency in PFT outcomes in frail CAD patients.

Methods: PFTs were performed 1–2 months after percutaneous intervention in consecutive frail CAD patients to determine safety and efficacy of the prescribed P2Y₁₂ inhibitors, in order to adapt therapy when risk of adverse events was assessed too high. Patients were considered frail if they had ≥ 3 known risk factors for adverse events. The therapeutic windows of the VerifyNow P2Y₁₂, Multiple electrode aggregometry (Multiplate) 6.5 M ADP (both Tantry, JACC 2013), and the light transmission aggregometry (LTA) 20 M ADP (Kerneis, JACC 2013) were used to classify patients as low, optimal or high PR. Inter-test reliability analysis using the quadratic weighted Kappa method was calculated to determine consistency in PR classification by PFTs.

Results: Classifying 50 patients in platelet reactivity categories resulted in consistent classification in 13 patients; 36 patients were classified in 2 PR categories and 1 patient in 3 categories. The inter-test reliability analysis showed slight to moderate agreement. LTA vs. Multiplate had a Kappa of 0.20; 52% were classified in the same category and 46% were classified in a higher PR group by the Multiplate. Verify-

Now vs. LTA had a Kappa of 0.27; 42% were classified in the same category and in 58% the LTA found optimal PR but the VerifyNow high or low PR. VerifyNow vs. Multiplate had a Kappa of 0.53; 56% were classified in the same category and 38% were classified in a higher PR group by the Multiplate.

Conclusion: Classifying CAD patients on P2Y₁₂ inhibitors in predefined PR groups showed slight to moderate agreement between PFTs. This hampers their usefulness in daily practice.

Disclosure of Interest: None declared.

PO057-MON

Platelet function testing in ACS patients using measurement of platelet P-selectin: comparison with other commercial tests

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Background: There are various platelet function testing methods available that can be used to monitor the effectiveness of antiplatelet agents in patients with cardiovascular disease. These include Aspirin Kits and P2Y₁₂ Kits (Platelet Solutions Ltd, UK) which are based on measurement of P-selectin on appropriately activated platelets. Following platelet activation in whole blood the test samples are treated with PAMFix, a patented fixing solution, and sent to a central laboratory for analysis by flow cytometry.

Aims: To compare the results of platelet function testing using different testing procedures in ACS patients treated with aspirin and clopidogrel ($n = 102$) or aspirin and prasugrel ($n = 56$) for at least 1 month.

Methods: We compared the kits from PSL with three other aspirin and four other P2Y₁₂ commercial tests: light transmission aggregometry (LTA), Multiplate, VerifyNow and Biocytex VASP. PAMFix treated P-selectin samples were analysed after storage for 3 and 28 days.

Results: Three of the aspirin tests used (P-selectin, LTA and VerifyNow) clearly identified 3 patients as being either non-responders to aspirin or non-compliant with therapy. Multiplate identified only 1 of these. All of the P2Y₁₂ tests demonstrated variability in the effectiveness of clopidogrel to inhibit platelet function. Prasugrel treatment resulted in significantly greater inhibition than clopidogrel. Three patients with very high P-selectin values were subsequently found to be not taking prasugrel and high values were also obtained using the four other P2Y₁₂ tests. Fixed P-selectin samples yielded identical results when analysed after 3 and 28 days.

Conclusion: The P-selectin tests were at least as effective in determining the inhibitory effects of antiplatelet therapies as compared with other commercial tests. The stability of samples for 28 days extends the possibilities for use of the P-selectin approach for remote platelet testing.

Disclosure of Interest: J. May Shareholder of: Platelet Solutions Ltd, N. Dovlatova Employee of: Platelet Solutions Ltd, A. Radhakrishnan: None Declared, S. Heptinstall Shareholder of: Platelet Solutions Ltd, S. Fox Shareholder of: Platelet Solutions Ltd.

PO058-MON

Efficacy of prasugrel administration immediately after percutaneous coronary intervention in acute myocardial infarction

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Background: Prasugrel, a potent thienopyridine, achieves better platelet inhibition than clopidogrel. However, the onset of effect is significantly delayed in patients with acute ST-elevation myocardial

infarction (STEMI), as hemodynamic stability and morphine application seem to exhibit significant influence.

Aims: We assessed the efficacy of prasugrel loading immediately after PCI for STEMI instead of preloading the unstable patient before revascularization.

Methods: We investigated 29 consecutive patients with acute STEMI (mean age 56 ± 2 years) admitted to our department who underwent primary PCI. Prasugrel efficacy was assessed by the platelet-reactivity-index (PRI; VASP assay) before and 1, 2, 4, 6, 12, and 24 h following an oral loading dose of 60 mg immediately after primary PCI. High on-treatment platelet reactivity (HTPR) was defined as PRI > 50%.

Results: During the observed period, prasugrel significantly and rapidly reduced platelet reactivity determined by PRI in acute STEMI patients ($P = 0.0002$). In detail, mean PRI ($\pm$ SD) was $70.5 \pm 12.4\%$ before (control) and $43.7 \pm 28.9\%$ at 1 h ($P < 0.001$ vs. control), $24.6 \pm 27.4\%$ at 2 h ($P < 0.001$ vs. control), $20.7 \pm 25.7\%$ at 4 h ($P < 0.001$ vs. control), $16.8 \pm 24.6\%$ at 6 h ($P < 0.001$ vs. control), $11.1 \pm 14.7\%$ at 12 h ($P < 0.001$ vs. control), and $8.8 \pm 8.9\%$ at 24 h ($P < 0.001$ vs. control) after loading. The HTPR rate was 48% at 1 h, 22% at 2 h, 15% at 4 h, 13% at 6 h, 4% at 12 h, and 0% at 24 h.

Conclusion: In contrast to previous reports describing a significant delay in onset of prasugrel-mediated P2Y₁₂ inhibition in acute STEMI, we observed a rapid onset with low HTPR rates comparable to those observed in stable non-STEMI or elective PCI patients. Prasugrel administered directly after primary PCI might therefore be a useful therapeutic strategy in patients with STEMI to provide strong and effective P2Y₁₂ inhibition.

Disclosure of Interest: A. Schäfer Speaker Bureau of: lecture fees Daiichi-Sankyo, Eli Lilly, U. Flierl: None Declared, J. Bauersachs: None declared.

PO059-MON

The antithrombotic effects of synthetic polypeptides derived from snaclecs: involvement of platelet glycoprotein VI antagonism

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Background: Currently prescribed antiplatelet drugs including cyclooxygenase inhibitors, ADP receptor antagonists, and glycoprotein (GP) IIb/IIIa receptor antagonists have one common side effect – an increased risk of hemorrhage and thrombocytopenia. On the contrary, deficiency in glycoprotein VI (GPVI) expression does not lead to severe bleeding.

Aims: To develop a series of novel hexapeptides (Tro α 6) and decapeptides (Tro α 10) derived from α subunit of trowaglerix, a potent specific GPVI-targeting snaclec, to become GPVI antagonists.

Methods: Using reverse-phase HPLC to obtain the sequence of α subunit of trowaglerix. Functional effect of synthetic polypeptides were examined by platelet aggregometer. *In vivo* studies include fluorescein sodium-induced venules thrombosis and ferric chloride (FeCl₃)-induced artery thrombosis.

Results: We found that Tro α 6/Tro α 10 specifically inhibited collagen-induced platelet aggregation through blocking platelet GPVI receptor, but not GPIIb or integrin α 2 β 1 receptors. In fluorescein sodium-induced platelet thrombus formation of mesenteric venules and FeCl₃-induced carotid artery injury thrombosis model, Tro α 6/Tro α 10 displayed prominent inhibitory effect of thrombus formation. In addition, Tro α 6/Tro α 10 significantly inhibited the platelet aggregation in response to collagen *ex vivo* without prolonging bleeding time. With the aid of computational peptide design and molecular dynamic simulations on the decapeptides and GPVI, possible binding site was identified near D1/D2 domain surface.

Conclusion: Synthetic hexa-/decapeptide, which interacted with GPVI and exhibited anti-thrombotic activity *in vitro* and *in vivo*, were identified as important peptide therapeutics for developing small-mass GPVI antagonists for arterial thrombotic diseases.

Disclosure of Interest: None declared.

PO060-MON

Correlation between platelet MRP4 expression and platelet function in patients under chronic aspirin treatment

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Background: Aspirin does not exert an equal antiplatelet action on all subjects. An important mechanism involved in drug resistances is platelet overexpression of Multidrug Resistance Proteins 4 (MRP4). We demonstrated that aspirin *in vivo* treatment (300 mg per die) enhances platelet MRP4 expression, after 15 days.

It was also demonstrated that inhibition of MRP4 mediated transport reduces platelet function.

Aims: To verify whether MRP4 expression increases in platelets obtained from patients under chronic aspirin treatment (100 mg per die) and whether these platelets are hyper-responsiveness.

Methods: We evaluated the changes on mRNA and protein MRP4 expression (Q-RT-PCR, Western blot, immunofluorescence and immunoelectron microscopy) and platelet aggregation in 2 populations: healthy volunteers (HV; $n = 100$) and aspirinated (100 mg per die) patients for more than 1 month (ASA; $n = 150$).

Results: In platelets obtained from ASA patients, it was found a statistically significant MRP4 enhancement of both mRNA and protein expression compared to HV (2.1 fold increase; $P = 0.002$ and 1.56 fold increase; $P = 0.025$ respectively) and it appeared mainly localized in plasma membrane.

Platelets obtained from ASA patients that present the highest levels of MRP4, belonging to the 4th quartile (from 0.022 to 0.36 $\Delta\Delta$ Ct), have significantly higher platelet aggregation in comparison with those belonging to the 1st–3rd quartiles (from 0.001 to 0.022 $\Delta\Delta$ Ct), in response to both ADP (2 μ M; 54 ± 16 vs. $40 \pm 28\%$ aggregation after 4 min, respectively) and collagen (2 μ g mL⁻¹; 51 ± 20 vs. $41 \pm 20\%$ aggregation after 4 min, respectively; 79 ± 40 vs. 94 ± 40 s for lag-phase, respectively).

Conclusion: We can assert that chronic aspirin treatment induces a platelet MRP4 overexpression and platelet with higher MRP4 levels are hyper-responsiveness.

These evidences suggest that platelet MRP4 overexpression identifies patients less sensitive to aspirin action.

Disclosure of Interest: None declared.

PO061-MON

Platelet function monitoring in patients on dual antiplatelet therapy undergoing urgent HIP surgery: preliminary results

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Background: In clinical practice, the number of patients on dual antiplatelet therapy (DAT) who undergo emergency surgery for femoral fractures is increasing. DAT contraindicates locoregional anesthesia because of an increased risk of bleeding. However, evidence of a resis-

tance phenomenon to treatment with aspirin and/or clopidogrel raises the possibility of ineffective inhibition of platelet adherence which could have implications during surgery. Platelet function monitoring with point-of-care methods opens new scenarios in the management of anesthetic techniques in these patients

Aims: To analyze preoperative platelet function by impedance aggregometry in a group of patients on DAT undergoing urgent femoral surgery.

Methods: Five patients (age 70–96 years) on DAT who underwent urgent femoral surgery at the Orthopedic Unit of the Padua University Hospital between March and June 2014 were enrolled. All patients gave informed consent. For each patient, a venous blood sample was drawn and 3 mL were collected in EDTA-containing tubes for determination of platelet count, while 2.7 mL were collected into sodium citrate 0.109 M-containing tubes for determination of PT, aPTT and platelet function tests using impedance aggregometry (Multiplate[®] Analyser, Roche). ASPItest and ADPtest were used to evaluate the effect of aspirin and clopidogrel on platelet function, respectively. The parameter considered was the area under the curve (AUC).

Results: Mean $\pm$ SD AUC for ASPItest and for ADPtest were 26.4 ± 17.6 (reference value 39–79) and 50.4 ± 30.1 (reference value 29–78), respectively, while platelet count and coagulation times were within normal limits.

Conclusion: The results of our preliminary study show that despite the continued use of DAT, *in vitro* resistance was demonstrated in 4/5 patients, on clopidogrel and 1/5 on aspirin. In patients on DAT undergoing emergency femoral surgery, platelet function testing may be a useful aid in establishing the actual bleeding risk.

Disclosure of Interest: None declared.

PO062-MON

Platelet reactivity in patients with acute myocardial infarction chronically treated with beta-blockers before the onset of the acute event

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Background: Catecholamines are known to increase during acute myocardial infarction (AMI) as well as the response to adrenergic stimuli. This may have an impact on platelet reactivity and modify the effect of the antiplatelet treatment.

Aims: a) To compare platelet reactivity of patients with AMI chronically treated with β -blockers before the onset of the acute event with those without this treatment and b) to assess if β -blockers influence the extension of myocardial damage.

Methods: We studied 526 patients with antiplatelet therapy within 48 h of the onset of AMI of which 96 were under chronic treatment with β -blockers (Ct- β B). Citrate anticoagulated blood (3.8%) was obtained at least 6 h after the administration of the antiplatelet therapy for platelet function studies: collagen ($1 \mu\text{g mL}^{-1}$), arachidonic acid (AA, 1 mM), ADP ($3.5 \mu\text{M}$) and Epinephrine ($5 \mu\text{M}$)-induced optical aggregometry (LTA); serotonin (^{14}C -5HT) release and TXA₂ synthesis in collagen ($1 \mu\text{g mL}^{-1}$)-stimulated whole blood and PFA-100. Troponin and CPK were used as markers of myocardial damage.

Results: Ct- β B patients had a significant decrease of collagen ($P < 0.002$) and AA-induced aggregation ($P < 0.05$), while no differences were found in LTA with ADP or Epinephrine. Collagen induced ^{14}C -5HT release and TXA₂ synthesis were reduced ($P < 0.05$) while the PFA-100 closure time was increased ($P < 0.05$) in patients under Ct- β B. Interestingly, the number of patients with $< 95\%$ inhibition of TXA₂ (vs. aspirin-free normal subjects) was also lower in patients under Ct- β B than in those without the chronic treatment: 14.6% vs.

29.8% ($P < 0.002$). Ct- β B patients had a significant reduction of troponin and CPK ($P < 0.05$).

Conclusion: Prior chronic treatment with β -blockers improves some effects of the antiplatelet therapy shortly after the onset of AMI and reduces the extension of myocardial damage.

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Disclosure of Interest: None declared.

PO063-MON

Proteomic profiling of platelet response to antiplatelet agents reveals a wide inter-individual variability and distinct signatures of drugs alone and in combination

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Background: Antiplatelet agents are a mainstay of therapy in acute coronary syndromes, yet cardiovascular events continue to occur despite the use of antiplatelet drugs alone or in combination, in part as the pharmacological response is highly variable and difficult to titrate. Proteomic profiling of platelet response to antiplatelet drugs provides an integrated read-out of several factors that may explain this.

Aims: We examined the response to antiplatelet drugs alone and in combination on platelet releasate following stimulation with thrombin in 3 healthy donors.

Methods: A P2Y₁₂ antagonist (PSB0739, 10 nM), a PAR-1 antagonist (RWJ56110, 10 nM) and aspirin (10 mM) were used at concentrations that blocked the platelet responses to their respective agonists assayed by light transmission aggregometry. Thrombin (1 U mL^{-1}) was used to stimulate secretion, a concentration that induced full aggregation of washed platelets even in the presence of drug. This recapitulates a limitation with platelet aggregation assays in assessing the response to drugs which makes it difficult to discriminate antiplatelet agents or combinations of drugs. The platelet releasate profile was determined by label-free quantitative mass spectrometry (nanoHPLC-Q Exactive MS and MaxQuant software).

Results: The profile of all proteins (> 1000) following the addition of the antiplatelet agents showed a strong donor clustering, indicating a wide inter-individual variability (protein 'barcode'), yet it was similar for the same individual in samples obtained weeks apart. There were also clear drug signatures with marked differences between individual drugs and between combinations of 2 or 3 drugs (270 proteins) despite no discernible differences in suppression of platelet aggregation.

Conclusion: Thus, profiling of platelet proteome better discriminates the effects of antiplatelet drugs than is possible with platelet aggregation and may help to better understand drug effect, to monitor drug response and possibly help to tailor therapy more effectively.

Disclosure of Interest: None declared.

PO064-MON

Dual antiplatelet therapy combining aspirin and ticagrelor in intracranial aneurysmal embolization. A 13-month French experience

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Background: Aspirin + clopidogrel is the most common dual antiplatelet treatment prescribed in neuroradiology units, but clopidogrel efficacy is unpredictable.

Aims: We thus evaluated the association aspirin+ticagrelor in a prospective group of 50 patients submitted to an intracranial stent procedure.

Methods: Fifty-one intracranial stent procedures for intracranial aneurysms (48 patients), intracranial arterial stenosis (1 patient) and venous sinus stenosis (1 patient) were performed from 12/2014 to 01/2015 in 50 patients.

Aspirine 160 mg was started 1 week before procedure and continued for 1 year. A charge dose of 180 mg ticagrelor was administered the evening before and the morning of the intervention. 90 mg ticagrelor were given the evening of the intervention, followed by 90 mg twice per day for 3 months.

Results: Twelve stent assisted coiling procedures, 37 flow diverter stents, 1 intracranial arterial stenosis and 1 venous stenosis treated by intracranial stent were included.

No death or intracranial hemorrhagic event was observed. 2 patients presented minor neurologic impairment in the first 24 h after intervention and MRI confirmed the presence of ischemic lesions. One patient did not receive the two 180 mg doses of ticagrelor. Another patient received one charge dose only the day of the intervention because the stent procedure was urgent. 4 patients developed femoral hematomas not requiring surgery.

Conclusion: Ticagrelor is a more efficient P2Y₁₂ inhibitor than clopidogrel, with short half-life allowing quicker platelet function recovery desirable in emergent interventions. However, little experience with ticagrelor in neurovascular procedures was available until now. In this cohort of 50 patients, no severe neurologic impairment was observed and only 2 cases presented small ischemic lesions with minor defect. In addition, only 4 nonsurgical femoral hematomas were observed. Further prospective trials are mandatory for confirming the good benefit/risk ratio of ticagrelor in this setting.

Disclosure of Interest: None declared.

PO065-MON

Inhibition of thioredoxin inhibits GPVI-mediated platelet activation

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Background: The role of thiol reductases and isomerases in thrombus formation is well-documented *post* platelet activation, and is dependent upon the NADPH/thioredoxin reductase/thioredoxin system. Inhibitors of this system are of clinical interest with regards to the treatment of inflammation and cancer, yet their effect on platelet activation is largely unknown. Considering the significant interest in using NADPH/thioredoxin reductase/thioredoxin inhibitors in the clinic, we thought it pertinent to determine their potential to affect haemostasis.

Aims: To evaluate the effects of thioredoxin reductase/thioredoxin inhibitors on platelet function.

Methods: Light transmission aggregometry was used to assess platelet aggregation in PRP. Fura-2-loaded washed platelets were used to examine agonist-induced Ca²⁺ release. Receptor cell surface levels, granule secretion and integrin αIIbβ3 activation were measured by FACS. Free thiols were evaluated with DyeLight 800.

Results: The level of free thiols on the surface of platelets was high, and was reduced by small molecule inhibitors of thioredoxin. These inhibitors (PMX 464 and PX-12) selectively reduced platelet responses to the GPVI-specific agonist, CRP-XL, while the protein disulphide isomerase inhibitor rutin had no effect. ADP-, thrombin- and U46619-induced platelet activation was unaffected by thioredoxin inhibitors.

Kinetics is a determining factor in the inhibition of GPVI-mediated aggregation responses by PMX 464, with increased incubation times leading to an enhanced inhibitory effect. In addition, early indications are that the effects of thioredoxin inhibition on platelet function extend to thrombus formation in whole blood under flow (type I collagen, Bioflux).

Conclusion: PMX 464 inhibits GPVI-mediated platelet activation and the formation of thrombi on collagen under flow conditions. These findings suggest that the use of thioredoxin inhibitors may affect haemostasis.

Disclosure of Interest: None declared.

PO066-MON

Prediction of high on-treatment platelet reactivity in clopidogrel-treated patients with acute coronary syndromes, using advanced artificial neural networks

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Background: The prospective GEPRESS (Gene Polymorphism, Platelet REactivity and the Syntax Score) study showed that in clopidogrel-treated patients with acute coronary syndromes undergoing percutaneous coronary intervention (PCI) and a SYNTAX Score (SS) > 14, high on-treatment platelet reactivity (HPR) at 1 month was an independent predictor of adverse cardiac events. Traditional statistical methods (TSM) do not predict HPR based on patients' characteristics. Artificial Neural Networks (ANNs) are computerized algorithms resembling interactive processes of human brain, which analyse very complex non-linear phenomena.

Aims: To evaluate 1) the correlation between platelet reactivity at 1 month and demographic, clinical and genetic variables of patients enrolled in GEPRESS, using TSM and ANNs; 2) the accuracy of ANNs in predicting HPR at 1 month.

Methods: A complete set of data was available from 603 patients. Forty-eight demographic, clinical and genetic variables were analysed by ANNs and TSM. Platelet reactivity was measured by the vasodilator stimulated phosphoprotein (VASP) phosphorylation assay at 3 time points (before PCI, at hospital discharge and 1 month after PCI) and expressed as platelet reactivity index (PRI). HPR was defined as PRI > 50%.

Results: The platelet reactivity phenotype was very unstable, as it changed in 43% of patients at the 3 time points. Using TSM, PRI at 1 month was inversely associated with diabetes mellitus and CYP2C19 681GG genotype, and directly associated with CYP2C19 681AA/AG genotypes (Spearman's test). ANNs identified additional variables associated with PRI at 1 month: SS, the number of diseased coronaries, history of coronary artery disease, CYP3A4, ITBG3, IRS-1 and ABCB-1 polymorphisms. Despite its better performance, the accuracy of ANNs in predicting HPR was only 53%.

Conclusion: Albeit superior to TSM, ANNs inaccurately predicted HPR, likely because HPR proved to be a highly unstable phenotype during the 1-month observation.

Disclosure of Interest: None declared.

PO067-MON

Aspirin resistance in medical inpatients on chronic aspirin treatment

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Background: High on clopidogrel platelet reactivity has been associated with an increased risk of major thrombotic complications in acute coronary syndromes (ACS) patients, whereas high on aspirin platelet reactivity is widely debated, and its definition and diagnosis are still uncertain.

Aims: To evaluate the response to aspirin by both methods measuring the entity of platelet inhibition and serum thromboxane B2 (TXB2) production in hospitalized patients on chronic aspirin treatment.

Methods: A total of 118 patients, admitted to an Internal Medicine Unit, and treated with aspirin (100–300 mg daily) for at least 6 months for primary or secondary prevention of cardiovascular disease, were enrolled. Aspirin effect was assessed 3 days after admission using two different Methods

serum TXB2 concentration measurement, and platelet aggregation induced by arachidonic acid (VerifyNow[®] aspirin reaction unit (ARU); cut-off of aspirin resistance ARU $\geq$ 550).

Results: Thirty-two patients (27.1%) had ARU values $\geq$ 550. ARU values were significantly related with TXB2 levels ($r = 0.61$, $P < 0.001$). Patients with ARU $\geq$ 550 had significantly higher levels of TXB2 with respect to those with ARU $<$ 550 (9.9 [1.9–30] ng mL⁻¹ vs. 0.9 [0.6–2.2] ng mL⁻¹; $P < 0.001$). At linear regression analysis erythrocytes sedimentation rate (ESR) was the only variable significantly associated with ARU values, whereas ESR, chronic kidney disease and dyslipidemia were significantly associated with TXB2 levels.

Conclusion: A high prevalence of aspirin resistance was documented in hospitalized patients on chronic aspirin treatment. The correlation between the functional assay and measurement of the pharmacological target demonstrates that platelet hyper-reactivity is related to an impaired effect of the drug. Aspirin resistance, documented by high levels of ARU and serum TXB2, might be associated with an inflammatory process not only in the acute phase of ACS, but also in other acute illnesses.

Disclosure of Interest: None declared.

PO068-MON

TXA2-binding proteins, dipetalodipin and triplatin, block platelet-mediated nets formation *in vitro* and inhibit thrombosis *in vivo* without impairing hemostasis

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Background: Thromboxane A2 (TXA2) is synthesized from membrane-released arachidonic acid during platelet activation and plays an important role in thrombus formation. It was recently demonstrated that platelet-derived TXA2 mediates the formation of neutrophil extracellular traps (NETs). Dipetalodipin and triplatin are salivary gland proteins that exhibit high affinity binding to prostanoids, such as TXA2 which results in a potent inhibitory effect of platelet aggregation *in vitro*.

Aims: In this study we evaluated the ability of dipetalodipin and triplatin to block NET formation *in vitro*. We also investigated the *in vivo* antithrombotic activity of TXA2 binding proteins by employing two murine models of experimental thrombosis.

Methods: Clotting times were monitored in human PRP or in PPP (supplemented with procoagulant phospholipids). Platelet-assisted NET formation was identified by confocal microscopy using antibodies against citrullinated histone. The antithrombotic effect was assessed, *in vivo*, by two models of arterial thrombosis.

Results: Dipetalodipin and triplatin abolished the collagen-mediated acceleration of human platelet-rich plasma clotting. However this effect was not observed in the absence of platelets. Remarkably, we observed that both inhibitors abolished the platelet-mediated formation of NETs *in vitro*. Dipetalodipin and triplatin significantly increased carotid artery occlusion time in a FeCl₃-induced injury model. Treatment with TXA2 binding proteins also protected mice from lethal pulmonary thromboembolism. Effective antithrombotic doses of dipetalodipin and triplatin did not increase blood loss.

Conclusion: Our results demonstrate for the first time that TXA2-binding proteins, dipetalodipin and triplatin, exhibit additional anti-hemostatic effect by decreasing NETs generation. Notably, both molecules inhibit arterial thrombosis without impairing hemostasis.

Disclosure of Interest: None declared.

Arterial vascular disorders – clinical I

PO069-MON

ADAMTS13 and the risk of myocardial infarction: an individual patient data meta-analysis

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Background: Low levels of ADAMTS13 have been repeatedly associated with an increased risk of ischemic stroke but results for risk of myocardial infarction (MI) are inconclusive.

Aims: To perform an individual patient data meta-analysis from observational studies investigating the association between ADAMTS13 levels and MI.

Methods: A one step meta-analytic approach with random effect was used to estimate pooled odds ratios (OR) and corresponding 95% confidence intervals (CI) adjusted for confounding. Analyses were based on dichotomous exposures, with the 5th and 1st percentile of ADAM-

TS13 antigen levels as cut off values. Quartile analyses, with the highest quartile as a reference category, were used to assess a graded association between levels and risk ('dose response'). Additionally, we assessed the risk of the combined presence of low ADAMTS13 and high von Willebrand Factor (VWF) levels.

Results: Five studies were included, yielding individual data of 1501 cases and 2258 controls. Low levels of ADAMTS13 were associated with myocardial infarction risk, with an OR of 1.89 (95% CI 1.15–3.12) for values below the 5th percentile vs. above, and an OR of 4.21 (95% CI 1.73–10.21) for values below the 1st percentile vs. above. Additional adjustment for VWF levels did not affect the estimates (OR 1.79, 95% CI 1.05–3.06 for the 5th percentile cut-off and OR 4.16, 95% CI 1.74–9.98 for the 1st percentile cut-off). Risk appeared restricted to these extreme levels, as there was no graded association between levels of ADAMTS13 and MI risk over quartiles. Finally, there was only a minor synergistic effect for the combination of low ADAMTS13 and high VWF levels (VWF levels above vs. below the 90th percentile OR 1.72, 95% CI 1.22–2.42; ADAMTS13 levels below vs. above the 5th percentile OR 1.77, 95% CI 1.00–3.25; for the combination of the two risk factors OR 3.17, 95% CI 1.18–8.63).

Conclusion: Low levels of ADAMTS13 are associated with an increased risk of MI.

Disclosure of Interest: None declared.

PO070-MON

Diverse effects of PAI-1 On vascular smooth muscle cells phenotype switch

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Background: Pharmacological PAI-1 (plasminogen activator inhibitor-1) has been shown to block both vascular smooth muscle cells (VSMCs) proliferation and migration in cell culture and vessel neointimal formation following injury *in vivo*. However, role of PAI-1 in VSMCs biology have not been investigated.

Aims: The purpose of this study was to determine molecular effects of PAI-1 on VSMCs.

Methods: The expression of VSMC phenotypic switching gene markers were measured by real-time PCR.

Results: We show that PAI-1 up-regulated the expression of serum response factor (*SRF*), a transcription factor in regulating VSMCs phenotypic modulation. Both the VSMCs differentiation marker genes smooth muscle α -actin (*SMA*), smoothelin (*SMTN*) and synthetic markers genes cyclin D1, 2 are all up-regulated by PAI-1 in VSMCs, whereas using other recombinant PAI-1 mutants with selective loss-of-function mutation we demonstrated that binding of PAI-1 to VN down-regulates the VSMCs synthetic markers genes, and has no effect on the VSMC differentiation marker genes. PAI-1 mutant with markedly reduced binding capacity for LDL receptor family members abrogate the effects of PAI-1 in the regulation VSMCs phenotype. Furthermore, pretreatment of RAP (receptor associated protein) and anti-LRP1 (LDL receptor-related protein 1) inhibited the phenotypic modulation effects of PAI-1, suggesting receptor family is required for PAI-1 regulation of VSMCs modulation.

Conclusion: Altogether, these data show diverse roles of PAI-1-VN binding and LDL receptor family in the control of the differentiated properties of VSMCs and suggest that the binding of VN is a key process for PAI-1 regulating phenotypic switch of VSMCs.

Disclosure of Interest: None declared.

PO071-MON

Retinal vessel calibres and haemostasis in black and white South Africans: the SABPA study

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Background: Retinal arteriolar narrowing associates with hypertension development and increased cardiovascular risk. Evidence on whether the retinal vessel calibres are related to the haemostatic system is limited, especially in the black hypertension-prone population with a high stroke incidence.

Aims: To investigate the relationships between haemostatic markers and retinal vessel calibres in black and white South Africans.

Methods: We performed a cross-sectional study involving 71 black (mean age, 58 years; 43% women) and 80 white (mean age, 51 years; 50% women) teachers with a central retinal artery calibre smaller than 150.6 MU. We determined ambulatory blood pressures, haemostatic factors (fibrinogen, von Willebrand factor, D-dimer, plasminogen activator inhibitor-1 and clot lysis time) and retinal vessel calibres (arteriolar-to-venular ratio, central retinal artery and vein equivalent).

Results: A total of 79% of the black, and 40% of the white teachers were hypertensive. The black population had higher fibrinogen ($P < 0.001$), D-dimer ($P = 0.039$) and plasminogen activator inhibitor-1 ($P < 0.001$) with narrower arterioles ($P = 0.024$) and wider venules ($P = 0.001$). In the black population the central retinal vein equivalent (CRVE) was positively ($\beta = 0.293$; $P = 0.024$) associated with fibrinogen, whereas in the white population the central retinal artery equivalent (CRAE; $\beta = -0.256$; $P = 0.016$) was negatively and CRVE ($\beta = 0.234$; $P = 0.021$) positively associated with von Willebrand factor. Furthermore, clot lysis time was negatively associated with the CRAE ($\beta = -0.390$; $P = 0.014$) in the black group and positively associated with the CRVE ($\beta = 0.275$; $P = 0.008$) in the white group.

Conclusion: In individuals with possible arteriolar narrowing, fibrinogen may play a role in venular widening in the black population with higher haemostatic and blood pressure levels, while vWF is linked to both arteriolar narrowing and venular widening in the white population.

Disclosure of Interest: None declared.

PO072-MON

Prognostic value of the CHA₂DS₂Vasc score in patients experiencing a myocardial infarction

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Background: CHA₂DS₂Vasc score is an independent predictor for adverse outcome in patients with atrial fibrillation (AF) but its role in patients experiencing a myocardial infarction (MI) is unknown.

Aims: To evaluate the role of the CHA₂DS₂Vasc score as predictor of adverse outcome in long term follow-up after MI.

Methods: Patients with a recent IM and no evidence of mitral valve disease admitted to a cardiac rehabilitation between July 2006 and December 2014 were evaluated. Baseline characteristics, risk factors, comorbidities, CHA₂DS₂Vasc score, exercise training risk class and medications were recorded at admission and AF, heart failure (HF), acute coronary syndrome (ACS), ischemic stroke (IS) and death in the

follow-up. Statistical analyses were performed with Chi-square test, student's t-test and Cox regression.

Results: Overall, 319 patients were included in the study: mean age 63 ± 11 years, 79% men. 289 patients underwent revascularization procedures: 219 percutaneous technique and 70 surgery. One-hundred-eighty-seven (57.7%) patients presented a CHA₂DS₂VASC score ≥ 3 . During the follow-up period (mean 3.2 ± 2.4 years) 72 adverse event occurred: 4.4% of patients developed HF, 8.8% AF, 3.1% IS, 7.8% ACS and 1.6% died. 24 patients with a CHA₂DS₂VASC ≥ 3 developed AF (13%) and 4 with a CHA₂DS₂VASC < 3 (2.9%); OR = 3.75, 1.29–10.86, $P = 0.015$. These outcomes more frequently developed in patients with a CHA₂DS₂VASC ≥ 3 (compared to CHA₂DS₂VASC < 3): HF 6.5% vs. 1.5%, IS 4.3% vs. 1.5%, death 2.7% vs. 0% and the composite of death and cardiovascular events (IS and/or ACS) 13.0% vs. 11.1% (not statistically significant). ACS recurrence occurred in 6.5% and 9.6% of patients with a CHA₂DS₂VASC ≥ 3 and a CHA₂DS₂VASC < 3 , respectively.

Conclusion: A CHA₂DS₂VASC score ≥ 3 was a predictor of AF in long term follow-up of patients experiencing a MI. A non significant association with other adverse outcome was also observed.

Disclosure of Interest: None declared.

PO073-MON

Genetic risk factors on atherothrombotic disease: comparison between two territories

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Background: Myocardial infarction (MI) and stroke represent the most important complications of thrombosis disease.

Aims: The aim of the study was to evaluate the association between the PIA1/A2 polymorphism in the IIIa glycoprotein gen and C677T polymorphism in the 5,10 methylenetetrahydrofolate reductase gen in young Mexican patients with MI or stroke in two independent case-control studies.

Methods: A total of 297 patients ≤ 45 years old with MI and 297 healthy subjects in the control group were included. The diagnosis of MI was based on an electrocardiogram, clinical data and laboratory. They were admitted to the Intensive Coronary Care Unit of the Cardiology Hospital, CMN in Mexico City. In a second study, 235 patients ≤ 45 years old with stroke and 235 healthy subjects age-gender matched were included. Diagnosis of stroke was considered after an acute focal neurological deficit with duration greater than 24 h and confirmed by brain-computed tomography or magnetic resonance. The study protocol was reviewed and approved by the Human Ethical Committee of the IMSS, and conforms to the guidelines of the 1975 Declaration of Helsinki. Informed written consent was obtained from all subjects before enrollment.

Results: There was a difference in the PIA1/A2 genotype distribution ($P = 0.001$) between MI and control group, but not in the PIA1/A2 genotype distribution ($P = 0.67$) between control and stroke groups. In contrast, there was a significant difference in the C677T genotype distribution ($P = 0.001$) between stroke and control group, but not in the C677T genotype distribution ($P = 0.60$), between MI and control group.

Conclusion: The allele PIA2 represented an independent risk for MI but not for stroke. In contrast, the C677T polymorphism was associated with increased risk for stroke but not for MI. Our results suggest a possible different role of genetics factors on atherothrombotic dis-

ease such as MI and stroke. In one territory may be associated, whereas in other may be protector.

Disclosure of Interest: None declared.

PO074-MON

Cardiological and thrombophilic risk profile in patients with ocular arterial occlusion

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Background: Retinal vascular occlusion is common causes of blindness and visual morbidity. The role of thrombophilia in the etiology of retinal artery occlusion has not been adequately clarified.

Aims: The aim of our study was to establish the prevalence of major and potential inherited and acquired thrombophilic risk factors in 85 patients (49 females, 36 males; age: 56.6 years; range 22–73) with ocular arterial occlusions. The control group consisted of 68 (36 females, 32 males; age: 55.8 years; range 20–72) healthy subjects, without any vascular, eye-related disease.

Methods: In all participants the prevalence of Leiden mutation (FV Leiden), prothrombin variant (20210 G/A mutation), Leu 34 polymorphism of the factor (F) XIII A-subunit, deficiency of protein C, S, antithrombin and antiphospholipid antibodies (aPL) were assessed. Also intima-media thickness (IMT) in carotid arteries were measured using ultrasonography.

Results: Elevated aPL antibody level (IgG > 10 GPL; IgM > 20 MPL) were detected in 15 of 65 patients (23%) and in 4 of 60 controls (6.6%). The incidence of FV Leiden, deficiency of protein C were significantly more common in patients suffered from ocular arterial occlusions ($P < 0.05$) compared to controls. Patients with ocular arterial occlusions had greater IMT than healthy subjects (0.91 mm vs. 0.72 mm; $P < 0.05$). There was no significant difference in the prevalence of deficiency of protein S, 20210 G/A mutation, FXIII Val34Leu polymorphism and antithrombin level between patients and healthy subjects. Among classic risk factors only hypertension, diabetes mellitus, hypercholesterolemia and cigarette smoking were significantly more frequent in patients suffered from ocular arterial occlusions in comparison to controls.

Conclusion: These results show that thrombophilia plays a much important role in the pathogenesis of retinal artery occlusions. According to these data, thrombophilia screening should be consider in preventing and treatment of such events.

Disclosure of Interest: None declared.

PO075-MON

A novel assay demonstrates procoagulant platelets are increased in patient undergoing coronary angiography with differential effects by anti-platelet and anti-coagulant therapy

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Background: Procoagulant platelets are a subset of activated platelets that support thrombin generation and have been implicated in athero-

thrombosis. Study of procoagulant platelets is hampered by lack of specific and sensitive marker. We have shown a novel marker GSAO identifies necrotic/procoagulant platelets in whole blood flow cytometry assay.

Aims: We postulated that platelets from patients with coronary artery disease (CAD) have heightened propensity to stimulus-induced procoagulant phenotype, which may be modified by antiplatelet agents (APA).

Methods: After informed consent, blood samples from 60 patients were collected during coronary angiography from radial/femoral and coronary arteries. Thrombin (T) or a combination of thrombin and collagen (T/C) were used to induce platelet procoagulant phenotype in diluted whole blood. Previously, we established a normal response profile in healthy controls. Linear mixed effects models were used to explore the effect of modifying factors on procoagulant platelets.

Results: Compared to controls, subjects undergoing coronary artery angiography had higher proportion of procoagulant platelets at baseline (4.1% vs. 0.4%, $P < 0.0001$) and in response to T/C (31.7% vs. 13.3%, $P < 0.0001$). Controls and patients without CAD showed a marked synergistic increase in procoagulant generation with dual agonist (T/C). In contrast, patients with angiogram-confirmed CAD reached maximal procoagulant potential with thrombin stimulation alone. Aspirin alone had no effect, however patients on dual antiplatelet therapy (DAPT) demonstrated reduced T/C-induced procoagulant platelets compared to no APA (OR 0.61, $P = 0.0295$). Patients on UFH showed marked reduction in procoagulant platelets regardless of the stimulus: T (OR 0.32, $P < 0.0001$) T/C (OR 0.38, $P = 0.0001$).

Conclusion: Platelets in patients undergoing coronary angiography showed increased procoagulant potential, which was favourably modified by DAPT but not aspirin alone. Novel necrosis marker, GSAO has potential as a biomarker in coronary disease.

Disclosure of Interest: None declared.

PO076-MON

Relation between invasive central pulse pressure and thrombogenicity

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Background: Hypercoagulability according to high thrombin-induced platelet-fibrin clot strength measured by thrombelastography (TEG) has been associated with ischemic event occurrences in coronary artery disease (CAD) patients. Aortic central pulse pressure (CPP) has been independently associated with the occurrence and extent of CAD in patients undergoing coronary angiography. The relation between hypercoagulability and CPP has not been demonstrated.

Aims: Our aim was to investigate the potential relation between parameters of CPP using invasive hemodynamic techniques and thrombogenicity in CAD patients.

Methods: In 371 consecutive patients, CPP waveform was captured at the ascending aorta using a pigtail catheter during coronary angiography procedure. We calculated CPP, pulsatility index (PI, the ratio of CPP to diastolic pressure) and fractional CPP (FPP, the ratio of CPP to mean arterial pressure). Thrombogenicity was assessed by TEG prior to angiography.

Results: Patients were divided into CAD with essential hypertension (EH) group and without EH group. Patients with EH had significantly higher thrombin induced platelet-fibrin clot strength (TIP-FCS) vs. patients without EH (66.1 ± 6.0 mm vs. 63.9 ± 5.7 mm, $P = 0.012$). Patients in the highest TIP-FCS quartile had higher CPP ($P < 0.001$), PI ($P < 0.001$) and FPP ($P < 0.007$). TIP-FCS in patients with CPP cutoff ≥ 50 mmHg was significantly higher than in patients with CPP cutoff < 50 mmHg (66.4 ± 5.8 vs. 63.9 ± 5.0 , $P < 0.001$). CPP ≥ 70 mmHg was identified as the matched point for hypercoagulability

(TIP-FCS > 69.0 mm) in female patients (area under curve: 0.70; 95% CI: 0.61 to 0.78; $P < 0.0001$), and CPP ≥ 57 mmHg was identified as the matched point for hypercoagulability in African-American patients (area under curve: 0.67; 95% CI 0.61 to 0.72; $P < 0.0001$).

Conclusion: This study suggests that invasive CPP, PI and FPP are associated with hypercoagulability measured by TEG. Clinical significance of this relation is unknown at this time and should be further studied.

Disclosure of Interest: None declared.

PO077-MON

Drug-eluting coronary stents generate lower level of cellular activation compared to bare metal stents in stable angina patients

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Background: Drug-eluting stents (DES) are considered as reliable tools for coronary stenting, however, the direct effect of these stents on platelet, leukocyte and endothelium function has not been fully explored and compared to that of bare metal stents (BMS).

Aims: Here we analyzed the level of different mediators of cellular activation after elective DES or BMS stenting that may indicate the development of clinical complications.

Methods: We recruited 49 stable angina patients: 28 were treated with BMS (Integrity[®], Medtronic), and 21 received everolimus-eluting stents (Xience Prime[®], Abbott). Citrated plasma samples were collected at 3 different time points: (i) prior to stenting, (ii) after the procedure within 24 h, and (iii) after 1 month under dual anti-platelet therapy. Platelet activation was followed via surface P-selectin by flow cytometry with sCD40L and PDGF levels. Activation of leukocytes was analyzed by sICAM-1. Levels of sVCAM-1, sE-selectin, sP-selectin and von Willebrand factor antigen (vWFag) were measured to evaluate the degree of endothelial injury. Response to aspirin and clopidogrel treatment was also tested.

Results: Increased platelet activation was detected regardless of stent type and anti-platelet medication even after 1 month. However, in DES but not in BMS, PDGF level was significantly decreased during the time period. Concentrations of activation markers of coronary endothelium were more elevated after BMS compared to DES implantation. Although stenting caused no stent thrombosis until the clinical end point of 6 months, there were 6 BMS patients displaying restenosis with significantly increased sCD40L (645 ± 358 vs. 355 ± 177 pg mL⁻¹) and sICAM-1 (228 ± 55 vs. 186 ± 51 ng mL⁻¹) levels after 1 month vs. those without complications. Finally, non-responsiveness to anti-platelet drugs did not influence the alteration in these markers.

Conclusion: DES implantation seems to be a safer coronary intervention based on the changes in different cellular activation markers compared to BMS.

Disclosure of Interest: None declared.

PO078-MON

Neutrophil extracellular traps in coronary artery thrombi in patients with acute myocardial infarction

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Background: Patients with an acute ST-elevation myocardial infarction (STEMI) routinely undergo primary coronary intervention (pPCI) with

thrombus aspiration. We hypothesize that specific characteristics of the arterial thrombus contribute to disease progression and impaired myocardial reperfusion after successful PCI. This is called 'no-reflow' and is an important determinant of prognosis of STEMI patients. Recent data (including our own observations) indicate that thrombi often contain highly thrombogenic neutrophil nuclear extra cellular traps (NETs).

Aims: We aimed to assess the appearance of NETs in the aspirated coronary artery thrombi in STEMI patients.

Methods: Seventy-two STEMI patients were enrolled who underwent a PCI and thrombectomy. Patients with past history of revascularization, kidney and liver failure, and known malignancies were excluded. Thrombi, collected during pPCI, were stained using hematoxylin eosin and resorcin fuchsin and by immunohistochemistry for myeloperoxidase (MPO) and DNA. NETs were defined as the co-localization of extracellular MPO and DNA.

Results: About 37.5% of all thrombi showed co-localization of MPO and DNA, indicating NETs. NETs were not observed in micro thrombi (< 0.5 mm). Previous pilot data indicate that all thrombi larger than 0.5 mm show presence of NETs. There was no preferred NETs location within the thrombus, varying from diffuse presence throughout the thrombus, accumulation at the borders or clustered in hotspots within the thrombus.

Conclusion: Coronary artery thrombi contain NETs that are randomly distributed throughout the coronary artery thrombus, but are not present in micro-thrombi.

Disclosure of Interest: None declared.

PO079-MON

Association between glucometabolic control and degree of hypercoagulability in patients with combined type 2 diabetes mellitus and coronary heart disease

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Background: Patients with combined type 2 diabetes mellitus (T2DM) and coronary artery disease (CAD) represent a high risk population regarding atherothrombotic events. Hyperglycemia with consecutive hyperinsulinemia is associated with a prothrombotic milieu and endothelial activation with increased release of both tissue factor (TF) and tissue factor pathway inhibitor (TFPI).

Aims: To investigate whether haemostatic markers were associated with glucometabolic control assessed by HbA1c, HOMA2 index, fasting glucose and insulin.

Methods: The study population ($n = 137$) consists of patients with CAD and known T2DM, taking part in the EXCADI-trial, a study primarily on the effect of exercise training on markers of atherosclerosis. The regional Ethics Committee approved the study and all patients have given written informed consent to participate. Venous blood samples were drawn in a fasting condition at study start and citrated plasma was stored frozen at -80°C until analyzed. Levels of TF, free- and total TFPI Antigen, pro-thrombin fragment 1 + 2 and D-dimer were determined by ELISA. The CAT assay was used to measure *ex vivo* thrombin generation.

Results: HbA1c was significantly correlated to free- and total TFPI ($r = 0.198$ and $r = 0.186$ respectively, $P < 0.05$, both). A significant correlation was also seen between fasting glucose and free- and total TFPI ($r = 0.252$ and $r = 0.279$, respectively, $P < 0.01$, both). Further, the levels of free- and total TFPI were significantly correlated to TF ($r = 0.217$, $r = 0.308$, respectively, $P < 0.01$, both). No significant correlations were observed between glucometabolic control and thrombin generation.

Conclusion: The results demonstrate a positive association between poor glycaemic control and levels of TFPI in patients with combined CAD and T2DM, potentially due to endothelial activation. The significant relationship between TFPI and TF might also indicate a compen-

sating mechanism in order to inhibit activation of TF-dependent pathway of coagulation.

Disclosure of Interest: None declared.

Atherosclerosis I

PO080-MON

Association of ABCB1 C3435T polymorphism with blood glucose levels and risk of coronary artery disease in Thais

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Background: ATP-binding cassette transporter 1 (ABCB1) is a transporter protein called P-glycoprotein (P-gp) that functions as the cellular efflux for transporting a variety of substances. Previous reports have revealed that *ABCB1* C3435T (rs1045642) polymorphism is associated with alteration in P-gp expression among different populations and ethnicities. Lower expression of P-gp has been proposed to reduce insulin secretion in pancreatic beta cells of rat model, which is the leading cause of type 2 diabetes mellitus (DM). Moreover, DM is in turn a risk factor for coronary artery disease (CAD).

Aims: To investigate the relationship between *ABCB1* C3435T polymorphism with blood glucose levels and the risk of CAD in Thai subjects.

Methods: A total of 252 Thai subjects were enrolled in the study including 88 CAD, 63 non-CAD and 111 healthy controls. The polymorphism of *ABCB1* C3435T was determined by polymerase chain reaction-restriction fragment length polymorphism techniques using 2 units of *Sau3AI* restriction enzyme.

Results: The genotype frequencies of CC, CT and TT genotypes between CAD and healthy controls were significantly different (37.5 vs. 24.3, 48.9 vs. 47.7 and 13.6 vs. 9.9%, respectively, $P = 0.024$). However, this was not observed when compared those between CAD and non-CAD. Fasting blood glucose levels in CAD with TT genotype were significantly higher than those in individuals with CT and CC ($P = 0.001$ and 0.018 respectively). Interestingly, the TT genotype revealed an increased risk for CAD compared to CC genotype in healthy controls (OR=3.1; 95% CI 1.4, 7.3; $P = 0.007$).

Conclusion: These findings suggested that the association of the *ABCB1* C3435T polymorphism with an increased risk of CAD may be modulated by its effect on blood glucose levels.

Disclosure of Interest: None declared.

PO081-MON

Understanding gene expression in coronary artery disease through global profiling, network analysis and independent validation of key candidate genes

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Background: The molecular mechanism underlying the patho-physiology of coronary artery disease is complex, involving multiple biological pathways that interact with each other and can be visualized in the form of a network.

Aims: The aim of the study is to analyze the global gene expression pattern in coronary artery disease (CAD) patients and to identify significant differentially expressed genes and pathways associated with the disease in a representative cohort of Asian Indians.

Methods: We initially performed blood transcriptomics profiling in 20 subjects, including 10 CAD patients and 10 healthy controls on the Agilent microarray platform. Data was analyzed by Gene Spring Gx12.5, followed by network analysis using David v.6.7 and Reactome databases. The most significant differentially expressed genes from microarray were independently validated by real time PCR in 97 cases and 97 controls.

Results: A total of 190 gene transcripts showed significant differential expression (fold change > 2, $P < 0.05$) between the cases and the controls of which 142 genes were up regulated and 48 genes were down regulated. Genes associated with inflammation, immune response, cell regulation, proliferation and apoptosis pathways were enriched while inflammatory and immune response genes were displayed as hubs in the network, having a greater number of interactions with the neighbouring genes. Expression of EGR1/2/3, IL8, CXCL1, PTGS2, CD69, IFNG, FASLG, CCL4, CDC42, DDX58, NFKBID and NR4A2 genes were independently validated; EGR1/2/3 and IL8 showed > 8-fold higher expression in cases relative to the controls implying their important role in CAD development.

Conclusion: Global gene expression profiling combined with network analysis can help in identifying key genes and pathways in CAD.

Disclosure of Interest: None declared.

PO082-MON

Insights into the genetic architecture of coronary artery disease in Asian Indians

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Background: Genetic approaches have helped in identifying a number of loci associated with coronary artery disease (CAD). Given that Asian Indians are highly predisposed to CAD at younger age could be attributed to a unique genetic architecture in this population.

Aims: We aimed to study the pattern of genetic association of a wide array of variants in a representative cohort of Asian Indians enrolled in the Indian Atherosclerosis Research Study.

Methods: We surveyed published literature and internal discoveries to prioritize single nucleotide polymorphisms (SNPs) that showed significant association with CAD. The selected variants were genotyped by Taqman assay in 500 CAD patients (includes cases with chronic stable angina [CSA] and myocardial infarction [MI]) and 500 age and gender matched controls. Significant physical interaction among proteins encoded by genes in these loci were analysed with *DAPPLE* (Disease Association Protein-Protein Link Evaluator). Gene enrichment analysis was performed using *ClueGO* to identify significant pathways.

Results: A total of 82 potential SNPs were selected. The most significant SNPs belonged to the well-established CAD risk locus, the 9p21.3 region (rs1333049, rs2383206, rs4977574), KIF6 (rs20455), CNM2

(rs12413409) and CELSR2-PSRC1-SORT1 gene cluster (rs599839). In subset analysis, in addition to 9p21.3 variants, there was significant association of PLA2G2A (rs3753827) and PHACTR1 (rs9349379) with MI and ZC3HC1 (rs11556924), HNF-1A (rs2259816) and CELSR2-PSRC1-SORT1 variants with CSA condition. The network generated by *DAPPLE* revealed 9 genes to be highly connected which formed two main clusters representing the lipid associated genes and cell-cycle regulating genes.

Conclusion: Genetic analysis on a representative Asian Indian cohort confirmed the association of certain previously reported variants and a distinct association pattern in CSA and MI cases. Network analysis revealed an important role for lipids, inflammation and cell cycle regulation pathways in CAD.

Disclosure of Interest: None declared.

PO083-MON

Circulating lymphocytes and monocytes as risk factors for coronary artery disease in Indian population

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Background: Inflammation and adaptive immune response is the key feature of coronary atherosclerosis and its clinical manifestation. Circulating monocytes and lymphocytes are known to contribute to the progression of atherosclerotic lesions from a stable to an unstable state. The relationship and risk association between the circulating leukocyte subpopulation and acute coronary syndrome has not been studied in detail.

Aims: We investigated the relationship between the circulating monocyte, and T lymphocyte subsets and patients with myocardial infarction compared to healthy controls in an Asian Indian population

Methods: The proportion of different monocyte population (CD14⁺⁺ CD16⁻, CD14⁺ CD16⁺ and CD14⁺ CD16⁺⁺) and T helper cells (CD3⁺ CD4⁺) with intracellular expression of IFN- γ , IL17 were assessed by Flow cytometry in the peripheral blood of patients with myocardial infarction ($n = 71$) and healthy controls ($n = 60$). Statistical analyses were performed using SPSS version 17.0 for Windows.

Results: The frequency of CD14⁺⁺ CD16⁻ inflammatory monocyte population, and the CD4⁺ IL17⁺ (Th17) were significantly higher in MI patients compared to control (69.04 ± 1.25 vs. 78.53 ± 1.27 $P < 0.001$ and 3.91 ± 0.19 vs. 4.69 ± 0.3 , $P = 0.03$ respectively). While the frequency of CD16^{high} monocytes with regulatory function was lower in MI patients (5.78 ± 0.46 vs. 4.10 ± 0.38 $P = 0.005$) We also observed a significant increase in the CD4/CD8 T cell ratio in the patient population compared to control (4.09 ± 0.28 vs. 4.55 ± 0.36 , $P = 0.037$). Logistic regression analysis revealed an age, gender and risk factor adjusted independent odds ratio of 1.35, 95% CI 1.05–1.74, $P = 0.018$ for Th17 cells and 1.12, 95% CI 1.06–1.18, $P < 0.001$ for inflammatory monocytes.

Conclusion: Our results suggest that inflammatory monocyte (CD14⁺⁺ CD16⁻ and the IL17 secreting pro inflammatory Th17 cells can be independent predictors of acute Myocardial infarction.

Disclosure of Interest: None declared.

PO084-MON

Translational informatics approach linking risk of coronary artery disease, infection and inflammation

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Background: Translational informatics approaches are needed for integrating diverse and ever accumulating data to enable translational medicine. Herein, we implemented a novel approach for understanding the link between infection and coronary artery disease (CAD) along with potential biomarkers for clinical use.

Aims: To develop a translational informatics approach to link molecular mechanisms and identify potential biomarkers.

Methods: We searched the CAD-gene database, Uniprot database for infection and inflammation, and Cytomegaloviruses (CMV) induced genes published by Wang *et al.* (2010). Clinical phenotypes were extracted from Unified Medical Language System and gene ontologies. Microarray data was obtained from GEO-dataset: GSE48060. The network construction was carried out using Cytoscape-3.0.2. Validation of results was performed in 200 patients with myocardial infarction (MI) compared with 200 unaffected controls from the Indian Atherosclerosis Research Study. Statistical analysis was carried out for logistic regression, Hosmer Lemeshow test and C-statistics using SPSS version-17.

Results: We identified 55 'Functional Communicator Ontologies' (FCOs) networking with 23 clinical phenotypes and 4 MI-specific FCOs were found in global gene expression analysis in CAD patients suggesting that, CMV infection can trigger xenobiotic mechanisms resulting in oxidative stress, inflammation and immune modulation. GGT-5 with all these molecular functions showed an odds ratio of 1.947 which improved to 2.889 after the addition of addition of CMV-Neutralizing antibody titers and antibodies to *Helicobacter pylori* and adjustment with conventional risk factors (CRFs). The C-statistics improved from 0.503 for CRFs to 0.699 for GGT in combination with above mentioned infections and CRFs.

Conclusion: Our translational informatics approach identified potential molecular mechanism of CMV infection in atherosclerosis and linking biomarker for risk prediction.

Disclosure of Interest: None declared.

PO085-MON

Identification of novel genetic mutations in low-density lipoprotein receptor (LDLR) gene causing familial hypercholesterolemia in Saudi Arab homozygous childrenAl-Allaf FA^{1,2,3}, Athar M^{1,3}, Abduljaleel Z^{1,3}, Taher MM^{1,3}, Bouazzaoui A^{1,3}, Abalkhail H⁴, Own R¹, Al-Allaf AF⁵, AboMansoor I¹, Azhar Z¹, Ba-hammam F³ and Alashwal A⁶

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Background: Familial hypercholesterolemia (FH) is an autosomal dominant disease and is a major risk factor for the development of coronary heart diseases (CHD). FH is predominantly caused by mutations in the low-density lipoprotein receptor (LDLR) gene. Mutations in apolipoprotein B (ApoB) or proprotein convertase subtilisin kexin 9 (PCSK9) can also lead to FH phenotype. Herein, we describe genetic

analysis of severely affected homozygous FH patients who are mostly resistant to statin therapy and are managed on an apheresis program.

Aims: To identify the genetic defects causing FH in Saudi population and to develop a diagnostic test to detect the involved mutations.

Methods: Blood samples were collected from 22 severely affected rare homozygous definite FH children patients. Additional 21 samples from their first-degree blood relatives have also been collected. Genetic screening for the LDLR, ApoB and PCSK9 gene mutations was performed by exon sequencing analysis using ABI 3500 genetic analyzer.

Results: We have identified two Novel mutations p.(D445*) and p.(G676Afs*33) at exon 9 and 14 of the LDLR gene respectively. The mutations are located in the EGF-precursor homology domain of the LDLR protein. We have also identified two known mutations p.(W577C) and p.(V806Gfs*11) at exon 12 and 17 of the LDLR gene respectively, and one known mutation p.(A53V) in exon 1 of the PCSK9 gene. Both the frameshift mutations and nonsense mutation could probably lead to either mRNA degradation or to a truncated protein that lacks important functional domains. Using a bioinformatics approach we theoretically explored the putative structure of the wild type and mutant LDLR protein and the implication of structural changes on the possibility of LDLR dimer formation.

Conclusion: This finding of common mutations causing FH in the Saudi population could serve to develop a rapid genetic screening procedure for FH. In addition, these data contribute to the understanding of the molecular basis of FH in Saudi Arabia.

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PO086-MON

Role of RS4988300 and RS3781590 LRP5 gene and RS1466535 LRP1 gene polymorphisms in the cohort of the prospective montignoso studySereni A¹, Giusti B¹, Sofi F¹, Saracini C¹, Galora S¹, Cario RD¹, Latta DD², Chiappino D², Abbate R¹ and Gensini GF^{1,3}

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Background: Atherosclerosis with its manifestations: coronary artery, cerebrovascular, peripheral arterial occlusive disease and abdominal aortic aneurysm (CAD, PAOD, CVD, AAA)] is a multifactorial conditions in which environmental and genetic factors are involved.

Polymorphisms in two members of the low density lipoprotein receptor (LDLR) family were demonstrated to be susceptibility factor for AAA: rs4988300 and rs3781590 in *LRP5* and rs1466535 in *LRP1* gene.

Aims: We aimed to evaluate the role of rs4988300 and rs3781590 *LRP5* and rs1466535 *LRP1* polymorphisms as determinants of atherosclerotic manifestations in the cohort of the prospective Montignoso study ($n = 1514$). In particular, we studied their association with PAOD, CAD, and glucose levels.

Methods: Genomic DNA was isolated from venous peripheral blood. We genotyped with TaqMan approach using the specific assays.

Results: At the multivariate logistic regression analysis, adjusted for traditional cardiovascular risk factors, the status of rs3781580 *LRP5* homozygous carrier was a significant and independent determinant of PAOD (OR = 2.44 [95% CI = 1.11–5.37], $P = 0.026$), and the status of rs3781580 *LRP5* carrier (heterozygotes+homozygotes) was a significant and independent protective factor of acute myocardial infarction

(AMI; OR = 0.54 [95% CI = 0.29–0.97], $P = 0.039$). Similar result was observed for the status of carrier of the rs4988300 polymorphism and AMI (OR = 0.55 [95% CI = 0.30–1.02], $P = 0.057$). Concerning the rs1466535 LRP1 polymorphism, no association was observed with atherosclerotic manifestations evaluated. Interestingly, the presence of rs4988300 LRP5 was associated with significantly ($P = 0.012$) reduced baseline glucose levels (GG = 100 [70–242], GT + TT = 98 [58–392]).

Conclusion: Our results indicated that LRPs polymorphisms might have different role in different arterial districts affecting, at least in part, the glucose metabolism.

Disclosure of Interest: None declared.

Blood coagulation tests I

PO087-MON

Active thrombin assay for improved treatment of thrombosis

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Background: Thrombin generation (TG) is a global assay of haemostasis that could monitor thrombosis risk, anticoagulation management, and guide therapy in bleeding disorders. However, TG methods currently available are not able to specifically measure active/free thrombin and rely on extrapolation from algorithms to account for thrombin bound to inhibitors such as alpha-2-macroglobulin (A2M).

Aims: To develop a substrate that measures true active thrombin.

Methods: Testing was performed using a patented substrate that allows for exclusive measurement of true active thrombin and does not react with thrombin bound to A2M and/or other thrombin inhibitors.

Results: Our substrate detects only free thrombin (Table) and functions in a chemiluminescent microplate assay, in plasma or whole blood. The presence of anticoagulants such as heparin, dabigatran and hirudin, inhibits the active thrombin generation. We have created luminogenic, fluorogenic and chromogenic versions of our substrate that was covalently linked to macromolecules in microplate wells. Our substrate reacted with free thrombin but not thrombin-A2M.

Substrate	Thrombin activity (Δ absorbance min ⁻¹)	
	Commercially available substrate	Our Active Thrombin Substrate
Thrombin	0.57	0.141
Thrombin-A2M	0.48	0.004

In summary, our microplate kit::

- Uses a fluorimeter or luminometer to directly measure free thrombin in plasma or whole blood.
- Covers concentrations of 9 orders of magnitude (no sample dilutions).
- Is amenable to change in detection substrates (i.e. electrogenic).
- Is sensitive to the presence of anticoagulants.
- Can be stored at room temp for rehydration at time of testing.
- Is compatible with all typical activating reagents (e.g. calcium, lipid, tissue factor).

Conclusion: Our patented substrate can be used in a stand-alone TG method, as an assay on existing laboratory analyzers or in point-of-care (POC) devices.

Disclosure of Interest: None declared.

PO088-MON

Accurate recovery of pegylated and non-pegylated therapeutic FVIII, using a specific aptt reagent, and application on an automated coagulation analyzer

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Background: PEGylated FVIII shows longer half-life than non-PEGylated FVIII, providing a longer protective period to hemophilic patients. In previous publications, pegylation seemed to demonstrate interference when assessed with APTT-based clotting assays, yielding variable and inconsistent results. A commercial instrument test system, capable of yielding consistent accurate results with both PEGylated and non-PEGylated FVIII, is essential in monitoring its therapeutic protection.

Aims: To demonstrate that Instrumentation Laboratory provides a singular laboratory solution for FVIII monitoring based on the ACL TOP Family automated instrument test system, utilizing SynthASil reagent with its specific FVIII application, to reliably yield accurate results for both PEGylated and non-PEGylated therapeutic FVIII in patients' plasma.

Methods: Samples were prepared spiking therapeutic FVIII into commercial FVIII-deficient plasma. Samples with PEGylated FVIII (N8-GP; Novo Nordisk) and non-PEGylated FVIII (Advate®; Baxter), ranged from levels 0.01 to 1.1 U mL⁻¹. Additionally run were (SSC/ISTH) Secondary FVIII Standards and known hemophilic plasmas. All samples were analyzed on the ACL TOP instrument using APTT SynthASil reagent after having calibrated the FVIII assay with HemosIL Calibration Plasma. The specific FVIII application designed for SynthASil was used throughout (Instrumentation Laboratory, Bedford, MA, USA).

Results: The ACL TOP FVIII SynthASil factor calibration was validated and saved. Results from all FVIII testing showed that for samples at all FVIII levels (0.01 U mL⁻¹ through 1.1 U mL⁻¹); the PEG-FVIII (N8-GP) consistently recovered at 90 ± 10%, comparable to the non-PEG-FVIII which recovered at 98 ± 7%.

Conclusion: Accurate results were seen for both therapeutic PEGylated and non-PEGylated FVIII therapeutics, (N8-GP & Advate® respectively) with excellent recovery, when analyzed on the ACL TOP instrument test system using the APTT-SynthASil reagent and its FVIII application.

Disclosure of Interest: None declared.

PO089-MON

Thrombin generation capacity and phenotypic association in ABO blood groups

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Background: Individuals with blood group O have a higher bleeding risk than non-O blood groups, and non-O blood types are overrepresented in patients suffering from thrombosis. It has been shown that

FVIII and von Willebrand Factor (vWF) are higher in non-O than in O blood groups, which could increase the conversion of prothrombin into thrombin and subsequently increase thrombin generation (TG).

Aims: We investigated the relationship between ABO blood group, TG and its two constituent processes: prothrombin activation and thrombin inactivation.

Methods: Plasma levels of vWF, FVIII, antithrombin (AT), fibrinogen, prothrombin and α_2 Macroglobulin (α_2 M) levels were determined. TG was measured in platelet rich (PRP) and platelet poor plasma (PPP) of 217 healthy donors. By a newly developed algorithm, prothrombin conversion and thrombin inactivation were obtained from overall TG.

Results: vWF and FVIII levels were lower (75% and 78%, $P < 0.001$) and α_2 M levels were higher (125%, $P < 0.001$) in the O group compared to the non-O group. Thrombin generation is 10% lower (ETP and peak) in the O group in PPP and PRP ($P < 0.05$). Less prothrombin was converted in the O group than in non-O groups (86%, $P < 0.05$). Thrombin decay capacity was lower in the O group as well (0.652 min^{-1} vs. 0.744 min^{-1} ; $P < 0.05$), and the balance between thrombin inhibitors AT and α_2 M was different between blood groups. In the O group, α_2 M plays a significantly larger role in the inhibition of thrombin than in the non-O group (126%, $P < 0.01$).

Conclusion: The lower TG in the O group is due to lower prothrombin conversion, and a larger contribution of α_2 M to thrombin inactivation. The former is unrelated to platelet function because it is similar in PRP and PPP, but can be explained by the lower levels of FVIII.

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PO090-MON

Haemostasis in cardiac arrest patients treated with therapeutic hypothermia

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Background: Therapeutic hypothermia improves neurologic outcome in patients resuscitated after out-of-hospital cardiac arrest, but hypothermia is suspected to increase the risk of bleeding. Therefore, patients with a suspected bleeding risk are not treated with hypothermia, although the influence on haemostasis is not fully investigated.

Aims: We aimed to investigate the haemostatic changes during therapeutic hypothermia. Our hypothesis was that clotting time was prolonged during hypothermia compared to normothermia.

Methods: We included cardiac arrest patients admitted to Aarhus University Hospital, Denmark, from January-August 2014. Informed consent was obtained from all patients or next of kin, and the Central Denmark Region Committees on Health Research Ethics approved the study. Patients were treated with hypothermia ($33 \pm 1^\circ\text{C}$) for 24–48 h. Blood was sampled both during hypothermia and at normothermia. Haemostatic changes were investigated by thromboelastometry (ROTEM[®]) using a sensitive low-tissue-factor assay, and by standard coagulation tests.

Results: We included 22 patients for data analysis. We compared samples obtained after 22 ± 2 h of hypothermia to samples obtained at normothermia approximately 48 h later. We found no significant changes in clotting time, clot formation time, time to maximum velocity and maximum velocity (all P -values > 0.17). Fibrinogen (functional) was above

the lower limit of the reference interval in all patients. The maximum clot firmness and fibrinogen (functional) were both significantly higher at normothermia compared to hypothermia (all P -values < 0.01). No significant differences were found in INR, aPTT or thrombin time comparing hypothermia to normothermia (all P -values > 0.10).

Conclusion: The present study showed no substantial haemostatic changes in cardiac arrest patients during therapeutic hypothermia when compared to normothermia.

Disclosure of Interest: None declared.

PO091-MON

Is TGA able to discriminate a different coagulation profile in inhibitor patients with severe hemophilia A as compared to non-inhibitor patients?

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Background: Patients with hemophilia A (HA) have impaired thrombin generation (TG) capacity and TG assay (TGA) values are linearly related to FVIII levels. A wide range of endogenous thrombin potential (ETP) values was reported in patients with the same FVIII activity. In the presence of FVIII inhibitors (INH), possible differences in the TG profile may exist but this has not been demonstrated so far.

Aims: This study was aimed at unravelling any difference in TG capacity in patients with severe HA with INH as compared to those without INH and at identifying which determinants are involved.

Methods: Patients with severe HA, of any age, with or without current INH, able to maintain a minimum wash-out period from treatment of 5 days were eligible. Blood samples were collected in a non-bleeding state. TGA was performed in platelet-rich and platelet-poor plasma $\pm$ corn trypsin inhibitor.

Results: TGA was performed in 102 patients with severe HA (median age 38 years; 87 without INH and 15 with high-responding INH) and in 69 age- and sex-matched healthy controls. TG was reduced in INH patients as compared to non-INH patients and the difference was statistically significant for ETP and thrombin peak under all experimental conditions. No correlation between TGA parameters and INH titers was found. F8 mutations were known in 88 patients (86%), being null in 50% and non-null in 36%. Null mutations were more prevalent in INH than in non-INH patients (80% vs. 45%; $P = 0.006$). TG was greater in patients with non-null than in those with null mutations and the difference was statistically significant for ETP and thrombin peak under all experimental conditions.

Conclusion: This study showed a greater TG impairment of in INH patients (irrespective of INH titers) and in patients with null mutations. Nonetheless, the ability of TGA to discriminate the presence of inhibitors and, ultimately, the bleeding phenotype in severe HA deserves further investigation.

Disclosure of Interest: None declared.

PO092-MON

Impact of edoxaban on a large panel of routine of more specific coagulation assays

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Background: Thanks to its predictable pharmacokinetics (PK) and pharmacodynamics (PD), biological monitoring of edoxaban is not required. Nevertheless, evaluation of plasma drug concentration may be valuable in specific situations.

Aims: To assess and provide good laboratory practise for the accurate estimation of edoxaban plasma concentrations using routine or more specific coagulation assays.

Methods: Edoxaban was spiked at increasing concentrations (0–1000 ng mL⁻¹) in pooled citrated normal human platelet poor plasma (PPP) to measure Prothrombin Time (PT), activated Partial Thromboplastin Time (aPTT), dilute Russell Viper Venom Time (dRVVT), Thrombin Generation Assay (TGA) and anti-Xa chromogenic assays.

Results: Depending on the reagent, PT was not sensitive enough to allow accurate quantitative measurement of plasma drug concentrations. Indeed, the 2xCT was 97 and 296 ng mL⁻¹ with the most and the worst sensitive reagents, respectively. The mean C_{max} obtained in phase-III trials was around 150 ng mL⁻¹. Thus, depending on the reagent, a normal PT cannot exclude the presence of edoxaban at therapeutic concentration. aPTT did not present a better sensitivity. dRVVT could be useful to assess the PD of edoxaban at C_{max} but is not sufficiently sensitive (2xCT of 70 and 82 ng mL⁻¹ for the screen and the confirm reagent, respectively) for C_{min} assessment which is around 20 ng mL⁻¹. Chromogenic anti-Xa assays were very sensitive (from 4 to 14 ng mL⁻¹). TGA may be useful to assess the PD of edoxaban but its turn around time and the lack of standardisation are limitations.

Conclusion: PT, depending on the reagent, and dRVVT could be used as screening test to assess (*supra*)therapeutic levels of edoxaban. Chromogenic anti-Xa assays using calibrators should be recommended to accurately estimate edoxaban concentrations.

Disclosure of Interest: None declared.

PO093-MON

Modification of a thrombin generation test to identify new cofactors of the protein C pathway in plasma

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Background: Most of the known risk factors for venous thrombosis (VT) are part of the anticoagulant protein C (PC) pathway or modulate its activity, although there may be unknown plasma factors that modulate this pathway. The Automated Calibration Thrombogram (CAT) is a test that measures the potential of a plasma to generate thrombin.

Aims: To develop a modified CAT assay to make it sensitive to changes in plasma concentration of new cofactors of the PC pathway and to analyze its association with the risk of VT.

Methods: Thrombin generation was analyzed in plasma from 251 patients with an idiopathic VT history and 303 healthy controls in the absence or presence of a mixture of activated PC (APC; 5 nM) and protein S (PS; 50 nM). Five parameters were analyzed: lag time (TL, min), endogenous thrombin potential (ETP, nM min), peak height (PK, nM), time to peak (TTPK, min) and initial slope (V₀).

Results: We observed higher values of PK ($P < 0.001$), ETP ($P = 0.0048$) and V₀ ($P < 0.001$) in patients than in controls. The addition of APC and PS increased the TL and TTPK values ($P < 0.001$), and decreased the ETP, PK and V₀ values both in patients and controls ($P < 0.001$). However, the increase in TL and TTPK and the decrease in PK was greater in controls than in patients ($P < 0.001$). Furthermore, 4 controls (2.6%) and 14 patients (7.2%) did not respond to the addition of APC/PS, while 11 controls (7.2%) and 5 patients (2.6%) responded excessively to the effect of the addition of APC/PS, taking as reference the average ± 1.5 SD ($P < 0.001$).

Conclusion: These results suggest that our modification of the CAT assay may be useful to identify individuals with an increased VT risk, and for the identification of new cofactors of the PC anticoagulant pathway. ISCIH (PI12/00027, RD12/0042/0029, PI14/00512, PI14/00079, FI14/00269, FIS-CP09/00065), FEDER, Generalitat Valenciana (Prometeo 2015), IIS La Fe (2012/0221). SN is an IIS La Fe researcher. PM is a Miguel Servet researcher.

Disclosure of Interest: None declared.

PO094-MON

Preliminary performance data of a new assay for the quantitative determination of the activity of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) using a single calibration curve for both types of heparin*

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Background: Heparin (UFH and LMWH) considerably accelerates the inactivation of thrombin and coagulation factor Xa by antithrombin. Therefore UFH and LMWH preparations are widely used as prophylactic and therapeutic anticoagulants. The quantitative determination of the activity of UFH and LMWH usually requires heparin type specific calibration, increasing the risk of calibration curve mix-up in clinical practice, causing additional work for their establishment and increasing material complexity.

Aims: Development of an automated chromogenic assay for the quantitative determination of the activity of UFH and LMWH in citrated human plasma using a single calibration curve for both types of heparin.

Methods: A ready-to-use Factor Xa-dependent chromogenic assay, a dedicated calibrator set and different levels of UFH- and LMWH-containing controls have been developed. Calibrator and controls are traceable to the International Standards for UFH and LMWH. Applications have been developed for different analyzers.

Results: The heparin assay is calibrated from 0.10 to 1.50 IU mL⁻¹ and shows linearity over the entire range. Plotting the activities of plasma samples spiked with the International Standards for UFH and LMWH against their nominal activities and performing linear regression results in slopes between 0.90 and 1.10, demonstrating excellent recovery. Equal nominal activities of different brands of UFH and LMWH result in comparable raw values, showing suitability of the

assay for a variety of heparins. Calibration stability is given for at least 3 months.

Conclusion: The new heparin assay in combination with calibrator and controls offers a reliable method for the quantitative determination of the activity of UFH and LMWH in citrated human plasma using a single calibration curve for both types of heparin.

*Under development. Not available for sale. Product availability may vary from country to country and will be subject to varying regulatory requirements.

Disclosure of Interest: M. Wilkens Employee of: Siemens Healthcare Diagnostics Products GmbH, B. Becker-Scheidemann Employee of: Siemens Healthcare Diagnostics Products GmbH, A. Borchert Employee of: Siemens Healthcare Diagnostics Products GmbH, S. Halilovic Employee of: Siemens Healthcare Diagnostics Products GmbH, A. Hecker Employee of: Siemens Healthcare Diagnostics Products GmbH, R. Hoffmann Employee of: Siemens Healthcare Diagnostics Products GmbH, I. Jennemann-Watzka Employee of: Siemens Healthcare Diagnostics Products GmbH, M. Muth Employee of: Siemens Healthcare Diagnostics Products GmbH, R. Neunziger Employee of: Siemens Healthcare Diagnostics Products GmbH, J. Patzke Employee of: Siemens Healthcare Diagnostics Products GmbH, A. Scheidler Employee of: Siemens Healthcare Diagnostics Products GmbH, S. Schmidt Employee of: Siemens Healthcare Diagnostics Products GmbH, B. Wecht Employee of: Siemens Healthcare Diagnostics Products GmbH, A. Lichte Employee of: Siemens Healthcare Diagnostics Products GmbH.

PO095-MON

Provision of external quality assurance for thromboelastography (TEG) and thromboelastometry (ROTEM)

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Background: There has been a marked increase in Point of Care testing (POCt) of haemostatic tests recently and one area showing growth is Thromboelastography/Thromboelastometry. Thromboelastography is performed with the TEG device whilst Thromboelastometry is performed with the Rotem device. Both methods measure the elasticity of a forming clot but measure different parameters and cannot be directly compared. Devices are sited in theatres and specialist wards and usually operated by non laboratory staff.

Aims: Following a pilot study UK NEQAS for Blood Coagulation has launched a formal EQA programme with an aim to assess and improve the quality of these tests

Methods: Two surveys have been performed by 33 TEG users and 24 Rotem users. For TEG the median values are calculated for R.K alpha angle and MA using a plain cup to assess clotting and a heparinised cup to detect the presence of heparin. For the Rotem median values are calculated for CT, CFT, angle, A10, A15 and A30. Users have been asked to perform inTEM, exTEM and hepTEM. These tests assess the intrinsic and extrinsic clotting factors and the presence of heparin. However only the R time for TEG and the CT for Rotem are performance assessed (scored). Out of range results are derived by the furthest 10% of results form the median value.

Results: For the first survey a normal sample was distributed and was expected to give normal plasma values. Precision of these tests varied between parameters with a range of 10–73% for TEG and 2–37% for Rotem. For the TEG method 10 users were out of range for the plain cup and 9 for the heparinised cup. For the Rotem 4 centres were out of range for the inTEM test and 3 for the exTEM test.

Conclusion: Our data demonstrates the need for EQA for these devices.

Disclosure of Interest: None declared.

PO096-MON

Anticoagulation assessment with prothrombin time and anti-Xa assays in real-world patients on treatment with rivaroxaban

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Background: Monitoring of anticoagulation with the direct factor Xa inhibitor rivaroxaban is considered unnecessary in a routine clinical setting. However, the assessment of its anticoagulant effect may be desirable in certain clinical situations.

Aims: We aimed to compare prothrombin time (PT) reagents and anti-Xa assays to liquid-chromatography mass-spectrometry (LC-MS/MS) measurements of rivaroxaban concentration in samples from patients on treatment with rivaroxaban for stroke prevention in atrial fibrillation (AF).

Methods: Citrate plasma samples were obtained from 30 randomly selected AF patients on uninterrupted treatment with rivaroxaban for a minimum of 1 month. The anti-Xa assays, Direct Xa Inhibitor (DiXa-I[®]) and Heparin LRT[®] (both Hyphen-Biomed, Neuville-sur-Oise, France) were performed for both wide (0–500 ng mL⁻¹) and low (0–100 ng mL⁻¹) calibrations of rivaroxaban. Two PT measurements were taken (Thromborel S, Siemens, Marburg, Germany and Normotest, Technoclone, Vienna, Austria). Measurements were compared to LC-MS/MS using correlation, linear regression, intra-class correlation and Bland-Altman analysis.

Results: In 30 patients (9 female) of median age 71.5 years and BMI 26.5 kg m⁻², rivaroxaban concentrations between 2.4 and 625 ng mL⁻¹ (median 82 ng mL⁻¹) were measured by LC-MS/MS. PT reagents were poorly sensitive for rivaroxaban concentrations ($r^2 = 0.52$ and 0.09). Anti-Xa assays DiXa-I ($r^2 = 0.95$) and Heparin LRT ($r^2 = 0.97$) were sensitive to rivaroxaban in all concentrations, but especially in low concentrations with low calibrations ($r^2 = 0.97$ and 0.98 , respectively). The highest agreement occurred between Heparin LRT and low rivaroxaban concentrations with a mean difference of -5.3 ng mL⁻¹ (limits of agreement -12.9 to 2.4 ng mL⁻¹).

Conclusion: Anti-Xa assays can indirectly determine the concentration of rivaroxaban for a wide range of concentrations in real-world patients. An interpretation of anti-Xa and PT measurements in treatment with rivaroxaban requires knowledge of the local reagents.

Disclosure of Interest: None declared.

PO097-MON

Global hemostasis assays thromboelastography and thrombodynamics evaluate hypercoagulation in children with hemolytic anemia

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Background: Children with hemolytic anemia (HA) are characterized by the 100-fold increased risk of thrombosis associated with high lactate dehydrogenase (LDH) activity. Yet, standard hemostasis assays show no changes in the patients' hemostatic state.

Aims: The aim of this study was to investigate the hemostatic state in children with HA using global hemostasis assays.

Methods: Forty four children (aged 6.3 ± 4.1 years) with β -thalassaemia major ($n = 15$), hereditary spherocytosis (HS) in steady-state ($n = 20$) and during hemolytic crisis ($n = 9$) and 82 healthy children (aged 7.4 ± 4.3 years) as a control group were enrolled in this study. The LDH activity and hemoglobin concentration (Hb) were measured to evaluate the permanent hemolysis in patients ($LDH > 200$ IU L⁻¹, $Hb < 90$ g L⁻¹). The angle α in thromboelastography (TEG), clot growth rate V in thrombodynamics assay (TD), activated partial thromboplastin time (aPTT) and prothrombin time (PT) were measured to evaluate hemostatic state.

Results: TEG and TD parameters were increased in children with HA compared to control group (43 ± 13 $\mu\text{m min}^{-1}$ vs. 31 ± 9 $\mu\text{m min}^{-1}$, $P < 0.05$ for α and 28 ± 3 $\mu\text{m min}^{-1}$ vs. 24 ± 2 $\mu\text{m min}^{-1}$, $P < 0.05$ for V) while aPTT and PT were not changed. TEG and TD parameters were increased during hemolytic crisis compared to steady-state HS (47 ± 15 deg vs. 39 ± 11 deg, $P < 0.05$ for α and 31 ± 4 $\mu\text{m min}^{-1}$ vs. 27 ± 3 $\mu\text{m min}^{-1}$, $P < 0.001$ for V). PT and aPTT were in reference range.

Conclusion: Global hemostasis assays TEG and TD are consistent with clinical data for thrombosis risks of patients with HA and show hypercoagulation which increases in parallel with LDH activity.

Disclosure of Interest: E. Seregina: None Declared, J. Kuzminova: None Declared, A. Poletaev: None Declared, A. Sepoyan: None Declared, F. Ataullakhanov Shareholder of: HemaCore LLC, Employee of: HemaCore LLC, N. Smetanina: None Declared, A. Balandina: None Declared.

PO098-MON

Clinically important differences in pharmacodynamic effects of rivaroxaban and apixaban

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Background: Rivaroxaban and apixaban are non-vitamin K oral anticoagulants (NOACs) which exhibit inter-individual variation in pharmacokinetics (PK). However, it is uncertain whether this translates into a significant alteration in pharmacodynamics (PD) in standard laboratory measures used in clinical practice (aPTT, PT) or in thrombin generation as assessed by calibrated automated thrombogram (CAT). This inconsistency may erroneously inform clinical decisions in patients who experience NOAC related haemorrhage or prior to urgent surgery.

Aims: To observe the PD changes in aPTT, PT and thrombin generation in patients taking rivaroxaban and apixaban.

Methods: Observational, retrospective study of patients with on rivaroxaban 20 mg daily or 15 mg bd ($n = 210$) and apixaban 2.5–5 mg bd ($n = 46$) and predicted to have reached steady-state PK. Venous blood samples were taken from 2 to 24 h after the last dose. Plasma levels were assessed using a known calibrator (Hyphen Biomedical and Diagnostica Stago) on the STAGO STA-R automated analyser. aPTT (Actin FSL) and PT (Thrombotrol-S) and CAT (Thrombinoscope BV) were compared.

Results: NOAC levels varied between 5.3–527.9 ng mL⁻¹ for rivaroxaban and 20–331.2 ng mL⁻¹ for apixaban. There was a stronger correlation between rivaroxaban concentration and aPTT ($R^2 = 0.42$) and PT ($R^2 = 0.604$) compared to apixaban ($R^2 = 0.07$ and $R^2 = 0.24$, respectively). In patients with a NOAC level > 100 ng mL⁻¹, 113/127 (89%) patients taking rivaroxaban had a prolonged PT compared to 7/14 (50%) patients taking apixaban. Peak thrombin generation was inhibited more strongly with rivaroxaban ($R^2 = 0.51$) when compared to apixaban ($R^2 = 0.17$).

Conclusion: Direct measurement of NOAC concentration is more reliable than aPTT or PT measurement to guide clinical decisions. Peak thrombin generation is the most sensitive measure on CAT of anti-Xa inhibition by rivaroxaban and to a lesser extent apixaban. CAT could be useful to assess thrombin generation in patients on NOACs requiring intervention.

Disclosure of Interest: R. Baker Grant/Research Support from: Dr. Baker has received funding for clinical trials from Biogen Idec, Boehringer Ingelheim, Bayer, Baxter Healthcare, Pfizer, Daiichi Sankyo, Astellas and CSL Behring, has participated in clinical advisory boards for Amgen, Biogen Idec, Baxter Healthcare, Boehringer Ingelheim, Bayer, Alexion Pharmaceuticals, and Pfizer, Research support from, Baxter Healthcare, Bayer, Bristol Meyer Squibb and Alexion Pharmaceuticals has received conference travel support from Amgen, Novo Nordisk, Baxter Healthcare and Alexion pharmaceuticals, R. Szollosi: None Declared, S. McGregor: None declared.

PO099-MON

Monitoring new oral anticoagulants (NOACs) using new-generation thrombelastography TEG® 6S System

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Background: New oral anticoagulants (NOACs) including direct thrombin inhibitors (DTIs) and Xa inhibitors offer major advantages over vitamin K antagonists. Although officially these agents do not require monitoring, bleeding and ischemic events occur raising concerns for over or under inhibition during NOAC therapy. However, no standardized test is currently available.

Aims: To evaluate the utility of new point-of-care thrombelastography system (TEG® 6S, Haemonetics Corporation, Braintree, MA, USA) to quantify anticoagulant effects of NOACs.

Methods: Anticoagulant effect was assessed in healthy volunteers ($n = 26$) on no medications and in patients with non-valvular atrial fibrillation on Xa inhibitors (rivaroxaban and apixaban; $n = 40$), DTIs (dabigatran; $n = 24$). Anticoagulant activity was measured using TEG® 6S (or CORA®, Coramed Technologies, Niles, IL). Reaction time (R) with an Ecarin and a Factor XA based reagent. The study included both trough and peak concentrations as demonstrated by the time from last dosage.

Results: Compared to healthy volunteers, patients on Xa inhibitors had significantly longer R times (1.3 ± 0.3 min vs. 4.3 ± 1.7 min, $P < 0.001$). Patients on DTIs had longer R for both Ecarin (1.5 ± 0.2 min vs. 3.7 ± 1 min, $P < 0.001$) and FXa (1.3 ± 0.3 min vs. 3.5 ± 1.0 min, $P < 0.001$) levels. R-time of > 2.0 min accurately identified patients on DTI and Xa inhibitors with 100% accuracy.

Conclusion: The automated TEG 6S system with its multichannel anticoagulant cartridge is a highly sensitive and specific test to assess the anticoagulant effects of available NOACs. Further investigations employing anticoagulation monitoring using this assay are warranted.

Disclosure of Interest: None declared.

PO100-MON

***In vitro* models of thrombosis using human whole blood under flow conditions**

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Background: In order to understand thrombotic mechanism various *in vitro* and *in vivo* methods of thrombosis are employed. There are a limited number of methods that are able to employ human whole blood for the investigation of mechanisms of thrombosis and for assessing efficacy of novel therapeutic agents.

Aims: The aim of this study is to develop an *in vitro* model of thrombosis that is able to investigate thrombosis in human whole blood under physiological flow conditions.

Methods: To develop this model a BioFlux EZ was employed. Bioflux 200 (EZ), 48 well plates were coated with 100 mg mL^{-1} collagen for 1 h at 37°C and blocked with 5% (w/v) BSA in a TBS buffer for 1 h at 37°C followed by 16 h at 4°C , before priming with TBS at 37°C . Whole blood is prepared (with the first 2 mL discarded) into 0.109M sodium citrate (1/10). Alexa-488 labelled fibrinogen is added to a final 5% volume to the whole blood for 1 h at room temperature. As a control for small molecule work the same volume of vehicle is added (1% DMSO). CaCl_2 is added to a final concentration of 20 mM just prior to the initiation of the experiment. The blood is passed through the plate at a pressure of 10 dyne cm^{-2} . A lumascop-600 is employed for visualisation of clot formation with two simultaneous channels. Videos are prepared using the Bioflux 200 software. Time to occlusion is noted from the video and still images.

Results: Using rivaroxaban at a final concentration between 0.1 and $50 \text{ }\mu\text{M}$ we are able to demonstrate dose-dependent prolongation of time to occlusion. These data demonstrate that this model may be suitable for screening novel anti-thrombotic agents prior to *in vivo* models of thrombosis. It is also possible to use other forms of coating to initiate thrombus development.

Conclusion: This study shows that an *in vitro* model of thrombosis can be employed for the screening of novel therapeutic agents and for examining mechanism of thrombosis. Ultimately, this method may reduce numbers of *in vivo* experiments required for thrombotic studies.

Disclosure of Interest: None declared.

PO101-MON

FXII618, a new factor XII inhibitor: validation for thrombin generation assay

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Background: Thrombin generation (THG) is a sensitive test for overall assessment of the clotting potential. Despite its common use in research labs, standardization of pre-analytic conditions is lacking preventing its use as clinical diagnostic test. A major drawback is the contact phase activation during blood collection and plasma processing that deeply affects THG triggered at low dose of TF.

Aims: To test a new synthetic peptide (FXII618) against FXIIa and its use in THG in comparison to CTI.

Methods: THG by means of the calibrated automated thrombogram was performed on human normal plasma.

Results: *In vitro*, FXII618 inhibits hFXIIa ($\text{Ki } 22 \pm 4 \text{ nM}$) and completely inhibits APTT and FXII activity in plasma at $40 \text{ }\mu\text{M}$. To evaluate the effectiveness of FXII618 in the FXII-inhibition in THG, we used ellagic acid (EA) as thrombin trigger in normal plasma treated with increasing FXII618 concentrations. FXII618 reduced the EA-induced THG in a dose-dependent manner, reaching a complete THG inhibition at $7.5 \text{ }\mu\text{M}$ ($> 90\%$ reduction of ETP and peak) similarly to CTI. Both FXII618 and CTI did not affect the TGA, once induced by high levels of TF, thus confirming their specificity for FXII. As the contact activation could induce THG in the absence of an extrinsic- or intrinsic-specific trigger, we evaluate the THG in not triggered plasma treated with/without FXII618 and CTI. FXII618 inhibited THG significantly stronger than CTI (lag time: 55.8 ± 3.7 and 38.7 ± 1.9 min, and peak: 60.3 ± 0.8 and $78.0 \pm 6.8 \text{ nM}$, respectively). The addition of FXII618 to whole blood soon after collection instead of on processed plasma completely block thrombin activation during 120 min. In low-TF THG, FXII618 reduced the ETP to a similar extent as CTI, but delayed the lag time more potently (lag time: 12.3 ± 0.3 and 8.0 ± 0.2 min, respectively).

Conclusion: The improved blockade of contact activation makes FXII618 an attractive reagent for THG. Additional experiments using pathologic plasma samples are on going in order to validate the use of FXII618 in THG.

Disclosure of Interest: None declared.

PO102-MON

PF4-R, a novel heparin neutralizing reagent suitable for automated coagulometers: comparison with heparinase in routine coagulation tests

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Background: A common source of pre-analytical error in the coagulation laboratory is heparin contamination. Removal of the heparin effect is essential for coagulation screening tests to be performed without interference. Methods for neutralizing heparin include cationic compounds such as protamine and hexadimethrine, and extracorporeal removal devices, each of which is associated with drawbacks that include treatment-induced anticoagulation or hypercoagulability. Today, the heparin-digesting enzyme heparinase is commonly used to remove heparin from samples. However, this method also shows interferences in routine tests and is not suited for automation on coagulometers.

Aims: To compare the performance of a novel, fast, automatable platelet factor-4 (PF4)-based heparin neutralizer (PF4-R) with heparinase

in the activated partial thromboplastin time (aPTT), prothrombin time (PT) and Thrombin Time (TT).

Methods: Testing was performed on 100 clinical samples with elevated aPTT and 50 samples from patients medicated with unfractionated heparin. Samples were treated in parallel with PF4-R and heparinase then tested. The difference in clot times (CT) between treatments for each sample was calculated ($\text{PF4-R}_{\text{CT}} - \text{heparinase}_{\text{CT}}$). Results are reported as the 95% confidence interval of the mean difference in CT.

Results: Both treatments gave similar aPTT and PT results with mean differences of -0.4 ± 0.6 s and -0.7 ± 0.1 s, respectively. Heparinase prolonged the TT by an average of 6.4 ± 0.9 s relative to PF4-R. To investigate the bias, samples that did not contain heparin were analyzed to determine the effect of each treatment compared to the untreated sample (baseline). On heparin-free samples, heparinase prolonged the TT by 4.9 ± 1.4 s relative to baseline, whereas the PF-R gave a mean difference of -1.1 ± 1.0 s.

Conclusion: PF4-R and heparinase show equivalent performance in the aPTT and PT. However, heparinase treatment prolongs the TT due to an interference that does not occur with the novel PF4-R neutralizer.

Disclosure of Interest: S. Raynard Employee of: Precision BioLogic, Inc., A. Sadeghi-Khomami Employee of: Precision BioLogic, Inc., Z. Hui Employee of: Precision BioLogic, Inc.

PO103-MON

Global assays do not predict bleeding phenotype in hemophilia A

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Background: There is a need for global assays to predict hemophilia phenotype and guide therapy, owing to the wide variability in bleeding rates among patients with similar factor VIII activity (FVIIIact).

Aims: The goal of this study was to determine the correlation between baseline FVIIIact and parameters of three global assays, clot formation and lysis assay (CloFAL), thrombin generation assay (STP) and thromboelastography (TEG), as well as the relationship of these test parameters to bleeding phenotype.

Methods: Clinical and laboratory data were extracted from the database of a consented inception cohort study of bleeding disorders. CloFAL and STP were performed as previously described (Goldenberg, Thromb Res 2005; Simpson, Thromb Res 2011). Whole blood TEG was performed using kaolin activation according to manufacturer's recommendations (Haemoscope, Niles, IL, USA) with the addition of TPA 50 nanogram mL⁻¹ whole blood. Individual global assay parameters were correlated with FVIII act and bleeding rate.

Results: Thirty nine patients with FVIIIact between 0.5 and 30 IU dL⁻¹ were studied. Only CloFAL coagulability index (CI) and TEG reaction (R) time were significantly correlated with FVIIIact ($P = 0.01$, 0.029 , respectively). Univariate associations with bleeding rate in the previous year included FVIIIact ($P = 0.048$), STP time to maximal thrombin ($P = 0.037$), TEG angle ($P = 0.146$) and TEG R time ($P = 0.194$). After adjusting for baseline FVIIIact, no global assay parameter significantly predicted bleeding rate and only STP time to maximal thrombin approached significance ($P = 0.101$). Results using a life-long annualized bleeding rate yielded equivalent results.

Conclusion: Certain global assay parameters, namely CloFAL CI and TEG R time, moderately predict FVIIIact. However, none of the global assays evaluated predicted bleeding rate. Further development of existing global assays or novel laboratory approaches are required to predict hemophilia phenotype.

Disclosure of Interest: None declared.

PO104-MON

Testing an *in vitro* model of laminar flow for use in large-scale aptamer screening

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Background: Anti-platelet drugs can effectively inhibit thrombosis, but they present a bleeding risk and require extensive time to take effect. Aptamers are single stranded nucleic acid molecules that directly inhibit protein function by binding to their targets. 'Antidotes' rapidly reverse aptamer activity by binding to and disrupting their structure, thus allowing tight control of aptamer-induced protein inhibition. Using aptamers to target platelet function is a promising alternative to current anti-thrombotic therapies.

Aims: CellixTM is an *in vitro* model of laminar flow that assesses thrombus formation by measuring platelet binding to a substrate. The purpose of this study was to validate Cellix as a way to quickly and affordably test the activity of three structurally related aptamers that target Von Willebrand Factor. Data obtained with Cellix was compared to efficacy data obtained with a mouse model of FeCl₃-induced thrombosis.

Methods: In mice, aptamers were tested at doses of 0.093, 0.19, 0.38, 0.75, 1.5, and 3.0 mg kg⁻¹. In Cellix, equivalent concentrations were added to human whole blood, which was then flowed through collagen-coated channels of a VenafluxTM microchip. Total surface area of bound platelets was measured and expressed as a percentage of the surface area of bound platelets from control blood.

Results: At least 0.38 mg kg⁻¹ of each aptamer was required to inhibit thrombus formation *in vivo*. In Cellix 0.38 mg kg⁻¹ reduced platelet binding > 60%. Additionally 0.093 mg kg⁻¹ reduced binding by 40%, and 3.0 mg kg⁻¹ reduced binding > 98%. Our data show that Cellix is an ideal model for determining aptamer efficacy because it allows generation of a complete dose-response curve, even with minimal doses.

Conclusion: Cellix has advantages over *in vivo* models because it uses flowing human blood, requires less aptamer, and allows rapid screening of multiple molecules at multiple doses. Overall, our data show that Cellix is a clinically relevant, time, and cost effective tool for aptamer screening.

Disclosure of Interest: None declared.

PO105-MON

Polyhedrocytes in whole blood clots of patients with type 2 diabetes

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Background: Polyhedral erythrocytes (polyhedrocytes) have been observed in whole blood clots and intracoronary thrombi (Cines DG et al. *Blood* 2014). Platelets and fibrin(ogen) largely contribute to this phenomenon in healthy people.

Aims: Since glycation of proteins, including those within cell membranes, affects thrombus structure, platelet function, and fibrin properties, we hypothesized that type 2 diabetes (DM) patients may form less polyhedrocytes in blood clots possibly due to more rigid erythrocyte membranes and/or tighter fibrin networks, which might require larger forces to contract blood clots.

Methods: We analyzed whole venous blood clots formed after 24 h at 22 °C by adding 1 U mL⁻¹ human thrombin and 5 mM calcium chloride to citrated blood samples obtained from 25 DM patients on low-dose aspirin and statins (median HbA1c of 6.3%, range 5.5–7.9%). The reference group represented apparently healthy volunteers. Clots were fixed in 2.5% glutaraldehyde and prepared for scanning electron microscopy. We calculated the area covered by various forms of ery-

thrombocytes and the contraction ratio defined as a difference between the area of native red blood cells (RBCs) and polyhedral erythrocytes within the clot.

Results: In DM patients polyhedrocytes were detected in 23 (92%) clots; a median surface of a native RBC within these clots was 26.6 μm^2 and that of a polyhedrocyte 19.5 μm^2 . The contraction ratio was 27% in diabetic patients and 35% in healthy controls ($P < 0.05$). The area of the clot covered by polyhedrocytes $> 10\%$ ($n = 19$) in DM patients was found to be associated with higher blood glucose, C-reactive protein, triglycerides, and RBC count (all $P < 0.05$), but not with fibrinogen or HbA1c. The contraction ratio was inversely associated with the RBCs distribution width ($r = -0.53$, $P < 0.05$).

Conclusion: Diabetic patients on aspirin are characterized by impaired polyhedrocyte formation in blood clots, which contributes to high risk of thromboembolism.

Disclosure of Interest: None declared.

Cancer and thrombosis / hemostasis I

PO106-MON

Activation of coagulation by antineoplastic drugs for lymphoid tumors

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Background: Combining vorinostat (Vor), L-asparaginase (L-asp), doxorubicin (Dox) led to improved response rates in the treatment of lymphoid tumors. However, deep vein thrombosis (DVT) has been noted as one of the most serious side effects with these drugs, and how these regimens cause DVT is unclear.

Aims: We investigated the procoagulant effects of antineoplastic drugs for lymphoid tumors, especially focusing on tissue factor (TF) and phosphatidylserine (PS).

Methods: We examined the procoagulant effects of pharmacological concentration of Vor, L-Asp and Dox, using a human vascular endothelial cell line EAhy926, a hepatocyte cell line HepG2, lymphoid neoplastic cell lines HUT78 (cutaneous T cell lymphoma), Molt4 (acute T-lymphoblastic leukemia) and Ramos (Burkitt lymphoma), respectively, as laboratory cell models.

Results: Vor, L-Asp and Dox induced exposure of PS and cell surface procoagulant activity (PCA) on lymphoid tumor cells. Furthermore, Vor and Dox induced PS exposure and PCA on EAhy926 cells. Expression of TF antigen on cell surface was induced by Dox in each cells, while TF mRNA remained unchanged. Secretion of antithrombin (AT) or protein C from HepG2 cells was reduced only by L-Asp.

Conclusion: Vor and Dox may induce PCA in vessels not only through apoptosis of tumor cells but through PS exposure and/or TF expression on vascular endothelial cells. L-Asp induces thrombophilic state by reducing secretion of anticoagulant proteins such as AT. Therefore, surveying procoagulant markers such as D-dimers, prophylactic anticoagulant strategies should be considered in these antitumor drugs-combination regimen.

Disclosure of Interest: None declared.

PO107-MON

Procoagulant tumor microenvironment as target for a TFPI-like molecule

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Background: Tissue factor (TF) overexpression and phosphatidylserine (PS) exposure on surface of tumor cells or in tumor stroma lead a procoagulant microenvironment. In parallel, the endogenous FT pathway inhibitor (TFPI) has antitumor activity by inhibiting TF. Recently, a TFPI-like molecule was identified through the transcriptome analysis of the salivary gland from the adult *Amblyomma cajennense* tick. The recombinant protein, named Amblyomin-X, is able to inhibit the activation of FX through the extrinsic tenase complex or in the presence of phospholipids. Also, this molecule has been shown antitumor activity both *in vitro* and *in vivo*. In contrast, it has no cytotoxic effects on normal cells or healthy animals. Thus, a molecular recognition of this molecule by tumor cells or tumor stroma through procoagulant features could be a reasonable hypothesis to explain differences in its cytotoxicity.

Aims: evaluate the role of the procoagulant tumor microenvironment in the mechanism of action of Amblyomin-X.

Methods: PS-exposure detection was carried out by flow cytometry in tumor cells (SK-MEL-28 and Mia-PaCa-2) and non-tumor cells (human fibroblast). Differential scanning calorimetry (DSC) was performed to verify the disturbance caused by Amblyomin-X in lipid vesicles with exposed PS. Western blot (WB) was performed to assess FT and FVII protein levels.

Results: PS was more exposed on the membrane of the tumor cells compared to human fibroblasts. A significant broadening of 1,2-dimyristoyl-sn-glycero-3-[phospho-L-serine] (DMPS) transition was observed in DSC, that clearly indicating Amblyomin-X-DMPS interaction. Regarding WB analysis, FT protein levels were higher in tumor cells than in non-tumor cells and FVII was detected only in tumor cells. In Amblyomin-X-treated tumor cells, FT protein levels were increased after 2 h of treatment.

Conclusion: Tumor cells display characteristics required for a procoagulant microenvironment, which could favor Amblyomin-X uptake by these tumor cells or tumor stroma.

Disclosure of Interest: None declared.

PO108-MON

Modelisation of the procoagulant properties of adenocarcinoma pancreatic cells (BXPC3) and breast cancer cells (MCF7) and analysis of their specific interactions with the coagulation system

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Background: Cancer related hypercoagulability involves a complex interplay between malignant cells and coagulation system but not all mechanisms are entirely understood.

Aims: In the present study we dissected the mechanisms responsible for the procoagulant activity of BXPC3 and MCF7 cells by thrombin generation assay (TG) in different relevant conditions.

Methods: Cells were cultured and adhered in 96-well plates. TG of normal platelet poor plasma (PPP) added in wells carrying cancer cells was assessed by the CAT assay in different conditions of reagents. The CAT was also performed in plasma samples selectively deficient in coagulation factors, in the presence or absence of specific anti-tissue factor antibody (anti-TF), or corn trypsin inhibitor (CTI). Furthermore alternatively spliced TF (asTF), TF activity (TFa) and cancer procoagulant (CP) were also assessed.

Results: The TFa and asTF were found in abundant amounts in BXPC3 cells compared to MCF7 cells. The CP levels were higher in MCF7 cells. The BXPC3 had a higher effect than MCF7 cells on TG enhancement. The anti-TF had more important inhibitory effect on TG triggered by BXPC3. The CTI had more pronounced inhibitory effect on TG triggered by MCF7. BXPC3 presented more procoagulant phospholipids than MCF7. TG enhancement by both BXPC3 and MCF7 was mediated by FVII and intrinsic tenase. In contrast FXII and FXI were more important for TG enhancement by MCF7 rather than by BXPC3.

Conclusion: The two cancer cell lines (BXPC3 and MCF7) express substantially different procoagulant properties. The mechanism of activation of blood coagulation by the BXPC3 is dominated by the TF pathway. MCF7 additionally to TF imply also FXII and CP in the enhancement of TG. The modelisation of the procoagulant properties of cancer cells and the analysis of the specific interactions with the coagulation system could be a promising approach for the comprehension of cancer induced hypercoagulability.

Disclosure of Interest: None declared.

PO109-MON

Effect of hypoxia on tissue factor pathway inhibitor expression in breast cancer cells

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Background: Venous thrombosis (VT) is associated with reduced survival of breast cancer patients. The abnormal coagulation is initiated mainly by tissue factor (TF), and TF pathway inhibitor (TFPI) plays an important regulatory role. Hypoxia is one of the most pervasive physiological stresses in solid tumors and plays an important role in malignant progression. Regulation of TF and TFPI by hypoxia may contribute to the critical step that controls cancer-related hypercoagulation, but the underlying mechanisms are not known.

Aims: To explore the regulation of TFPI under hypoxic conditions in breast cancer cells.

Methods: MCF-7 and SK-BR-3 cells were cultured in 1% oxygen tension. Quantitative RT-PCR, ELISA, Western blot, promoter-luciferase reporter assay and ChIP assay were applied in this study.

Results: In comparison with normoxia, a significant decrease of TFPI mRNA and protein was detected under hypoxia along with an increase of TF protein, which was paralleled by the induction of HIF-1 α protein in MCF-7 and SK-BR-3 breast cancer cells. The down-regulation of TFPI was abolished when HIF-1 α was inhibited by HIF1 inhibitor (CAS 934593-90-5). A negative regulation of TFPI promoter (–1223 to +45 bp) luciferase activity was observed in response to hypoxia. Overexpression of HIF-1 α strongly repressed the activity of the TFPI promoter. We identified a HIF-1 α responsive region between –1223 and –545 bp of the TFPI promoter. ChIP assay indicated a direct binding between the HIF-1 α and the TFPI promoter, and further restricted the HIF-1 α responsive region to the region from –1157 to –1053 bp.

Conclusion: This study provides evidence that HIF-1 α is involved in the regulation of the procoagulant status of breast cancer cells through transcriptional regulation of the TFPI gene. These results suggest that thrombosis in breast cancer patients may correlate with local hypoxic regulation of coagulation factors and their inhibitors.

Disclosure of Interest: None declared.

PO110-MON

The thrombotic potential of circulating tumor microemboli

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Background: Thrombotic events can herald the diagnosis of cancer, preceding any cancer-related clinical symptoms. Patients with cancer are at a 4–7 fold increased risk of suffering from venous thromboembolism (VTE). However, the physical biology underlying cancer-associated VTE remains poorly understood. Several lines of evidence suggest that the shedding of tissue factor (TF)-positive circulating tumor cells (CTCs) from primary tumors may serve as a trigger for cancer-associated thrombosis.

Aims: To investigate the potential direct and indirect roles of CTCs and circulating tumor microemboli (CTMs) in VTE and to characterize thrombin generation by CTCs; to evaluate the contributory effect of platelet activation to the initiation and propagation of cancer-induced venous thrombus *in vitro*.

Methods: We performed clinical, biophysical and mathematical studies of single CTC and CTC aggregates from a patient with breast and lung cancer. We employed microfluidic devices to elucidate mechanism(s) of CTC-endothelial cell and/or CTC-platelet interaction under fluid shear flow.

Results: CTCs, ranging from single cells to aggregates comprised of 2–5 cells, were isolated using the high definition CTC assay and biophysically profiled using quantitative phase microscopy. Our numerical model demonstrated that CTMs could potentiate occlusive events that drastically reduce blood flow and serve as a platform for the promotion of thrombin generation in flowing blood. In microfluidic assays we observed an upward trend in rolling velocity as the number of cancer cells/cluster increased. Current studies are focused on defining the role of platelet activation in mediating CTM recruitment to EC and subsequent cancer-driven thrombus formation.

Conclusion: These results provide a characterization of CTM dynamics in the vasculature and enhance our understanding of CTCs and their potential direct and indirect roles in VTE. Future efforts will determine how CTCs heterotypic interaction with platelets can contribute to the vascular disease.

Disclosure of Interest: None declared.

PO111-MON

Fibrin degradation by RTPA improve nanoparticle drug delivery for tumors

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Background: Nanoparticles drug delivery system based on enhanced permeability and retention (EPR) effect for tumor drug delivery has

achieved some success. However, the EPR effect is not unique in all tumors and the EPR effect-dependent drug delivery is always complicated by the complex tumor microenvironment. As an important component of tumor extracellular matrix, fibrin is always abundantly sited near tumor vessels due to tissues factor highly expressed by tumor cells and the arrival of other coagulation factor by the leaky tumor vessels. Therefore, we propose fibrin in tumors might compress tumor vessels, reducing tumor blood flow, and accordingly compromising drug delivery for tumor.

Aims: We try to use a clinical widely used anti-thrombus drug recombinant tissue plasminogen activator (rtPA) to disrupt fibrin deposition in tumors and assess the effect of fibrin depletion on tumor blood flow and nanoparticles distribution in tumor.

Methods: Tumor-bearing mice models received i.p. administration of rtPA for 2 weeks with the daily dose of rtPA 25 mg kg⁻¹. RtPA tolerance was tested by monitoring coagulation function analysis, appearance of petechia or ecchymosis and the body weight or tumor size of mice models. Fibrin distribution was investigated by immunofluorescence staining. Blood flow in tumors was assessed by FITC-lectin labeling experiment. Nanoparticles were developed by an emulsion/solvent evaporation technique and nanoparticles accumulation in tumors was evaluated by *in vivo* imaging.

Results: There were no obvious adverse effects associated with the rtPA treatment in the present study. Fibrin was successfully disrupted by rtPA treatment. Functional vessels reflecting blood flow in tumor was increased about two fold more than control group. Results of *in vivo* imaging showed nanoparticles distribution in tumor was improved about 2.5 fold higher than control group.

Conclusion: RtPA treatment could destroy fibrin in tumors, improve tumor blood flow and enhance nanoparticle accumulation in tumor.

Disclosure of Interest: None declared.

PO112-MON

Human angiosarcoma cells express tissue factor and generate thrombin in a tissue factor-dependent manner

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Background: Angiosarcoma (AS) is a rare, highly aggressive and metastatic endothelial cell tumor that is associated with increased incidence of disseminated intravascular coagulation (DIC) in people. Tissue factor (TF) is a small transmembrane glycoprotein constitutively expressed on perivascular fibroblasts. The primary role of TF is to activate coagulation. Aberrant expression of TF on cancer cells can manifest clinically as paraneoplastic thrombosis in human patients with cancer.

Aims: To determine whether AS cells express TF and are procoagulant *in vitro*.

Methods: A cell line derived from a cutaneous angiosarcoma on the scalp (ASM-5), a kind gift from Dr. Antonescu, Memorial Sloan Kettering and non-neoplastic human umbilical vein endothelial cells (HUVEC) were examined for TF mRNA and antigen expression by quantitative reverse transcriptase PCR and flow cytometric analysis with a murine monoclonal TF antibody. Procoagulant activity was measured as lag time and area under the curve (AUC) generated from calibrated automated thrombography using human replete or specific coagulation factor (F)-deficient plasma and enzymatic inhibitors.

Results: ASM-5 cells expressed more TF mRNA and surface antigen than HUVECs. ASM-5 cells generated significantly more thrombin than HUVECs in human replete plasma. Thrombin generation in ASM5 cells was cell number dependent and was significantly reduced and abolished in human FVII- and FX-deficient plasma, respectively. Thrombin generation in ASM5 cells was unaltered by the addition of corn trypsin inhibitor, a FXII inhibitor.

Conclusion: The human angiosarcoma ASM-5 cell line expresses procoagulant functional TF. TF expression on tumor cells could explain the high incidence of hemostatic dysfunction in patients with angiosarcoma. TF on cancer cells could serve as a biomarker for potential thrombotic disorders and potential therapeutic target for angiosarcoma.

Disclosure of Interest: None declared.

PO113-MON

Role of autoantibodies to plasminogen at oncologic diseases

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Background: The binding of plasminogen (Pg) to cell receptors and extracellular ligands facilitates its activation to plasmin, which stimulates the extracellular matrix degradation, neoangiogenesis and tumor invasion. IgG associated with extracellular tissues, may also have the potential for Pg surface localization.

Aims: Determining the level of autoantibodies to Pg in plasma of patients with malignant new formations.

Methods: Methods of 2D-electrophoresis and MALDI-TOF mass-spectrometry were used for comparative research of proteins in plasma of patients with the prostate cancer (PC, *n* = 25), benign prostatic hyperplasia (BPH, *n* = 15), lung cancer (LC, *n* = 17), ovarian cancer (OC, *n* = 15) and mammary gland cancer (MGC, *n* = 29). Plasma of healthy donors (*n* = 29) was used as control. All patients signed informed consent for participation in this study. The levels of IgG and IgA autoantibodies to Pg were determined by ELISA. Affine chromatography on Pg-sepharose was used for the quantification of antibodies. The program ATTESTAT was applied for statistical treatment of results.

Results: 2D-electrophoresis revealed marker specific spots in the plasma of patients with PC as compared to control plasma. These marker spots were identified as pieces of Pg and immunoglobulin. When using ELISA elevated levels of IgG autoantibodies to Pg in plasma were detected in 33% of patients with BPH, 68% of patients with PC, 59% of patients with LC, 74% of patients with OC and 69% of patients with MGC, and only 12% of healthy women and 10% of healthy men. The frequency of occurrence of raised level of IgA autoantibodies to Pg was 72% for PC, while in the control and other types of cancer do not exceed 20%. The quantification of antibodies in plasma samples showed that the quantity of IgG to Pg in patients with PC was 27% from the total amount of IgG, and in healthy people – 9%.

Conclusion: Autoantibodies to Pg can serve as a marker for early diagnostics of oncologic processes.

Disclosure of Interest: None declared.

PO115-MON

Prothrombotic risk associated with intravenously given high dose vitamin C: another evidence warning against the blind faith in vitamins?

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Background: Potential risk of high-dose vitamin C consumption is being frequently ignored. Recently, gram doses of vitamin C are being used intravenously for cancer treatment, where extremely high concentration of vitamin C is directly exposed to red blood cells (RBCs) that can actively participate in thrombosis through procoagulant activation.

Aims: Here we examined the procoagulant and pro-thrombotic risks associated with intravenously given vitamin C.

Methods: We evaluated if vitamin C affects prothrombotic activation of RBCs using freshly isolated human RBCs. To identify the underlying mechanism, we investigated the alteration of intracellular level of reactive oxygen species, protein thiol, ATP and calcium. To evaluate thrombogenic effects of vitamin C, we introduced venous thrombosis animal model.

Results: Vitamin C (0.5–5 mM) increased procoagulant activity of RBCs via externalization of phosphatidylserine to outer membrane. Formation of PS-bearing microvesicles also increased. PS exposure was induced by the dysregulation of key enzymes in the maintenance of membrane phospholipid asymmetry, which was caused by vitamin C-induced oxidative stress, and resultant disruption of calcium and thiol homeostasis. Indeed, the intravenous administration of vitamin C (0.5–1.0 mg kg⁻¹) in rats *in vivo* significantly increased thrombosis. Notably, the prothrombotic activation by vitamin C was more pronounced in RBCs isolated from cancer patients, who are with higher risks of thrombotic events.

Conclusion: Vitamin C induced procoagulant and prothrombotic activation of RBCs, that lead to increased thrombosis *in vivo*. RBCs from cancer patients showed more pronounced sensitivity to vitamin C-induced prothrombotic activation, reflecting that intravenous gram dose vitamin C therapy needs to be carefully revisited.

Disclosure of Interest: None declared.

PO116-MON

Doxorubicin-induced MDR1/P-GP in MCF-7 breast cancer cells was associated with tissue factor overexpression and thrombin generation

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Background: Acquisition of properties rendering cancer cells resistant to the chemotherapy agents is among the main causes of treatment failure. Epithelial carcinoma breast cancer cells MCF7 are usually sensitive to several chemotherapeutic agents, but they can develop chemoresistance after prolonged exposure to cytostatic drugs, acquiring a more aggressive phenotype.

Aims: We investigate if the induction of chemo-resistance phenotype by MCF7 cells, documented by MDR1-Pgp expression, is associated with an enhancement of their procoagulant capacities.

Methods: Pre-treatment of MCF-7 cells for several weeks with increasing concentrations of doxorubicin, renders them chemo-resistant. Tissue factor (TF) and MDR1-Pgp expression by MCF7 cells were assessed by flow cytometry and western blot assays. Reverse transcriptase – polymerase chain reaction (RT-PCR) for TFmRNA and electrophoretic analysis in agarose gels was also performed. TF activity (TFa) from cancer cells was measured with a clotting based assay (Diagnostica Stago). Thrombin generation of normal platelet poor plasma (PPP) in the presence of MCF7 cells was assessed with the CAT assay (Diagnostica Stago).

Results: The acquisition of the chemo-resistance phenotype by MCF7 was correlated with a significant acceleration of thrombin generation. Chemo-resistant MCF7 cells expressed higher amounts of TF and showed marked TFa activity as compared to non resistant cells. Expression of TF by chemo-resistant MCF7 was correlated with the expression of the MDR1/P-gp.

Conclusion: Our results demonstrate that the acquisition of the chemo-resistance phenotype by the breast cancer cells is associated with enhancement of their procoagulant properties which are principally mediated by the TF expression.

Disclosure of Interest: None declared.

PO117-MON

Activated protein C up-regulates the ovarian cancer cell migration and promotes unclottability of cancer peritoneal fluid

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Background: We demonstrated previously the expression of EPCR in a large number of cancer cell lines in solid tumors and malignant hemopathies.

Aims: Study of the role of aPC, known to be a physiological anticoagulant, in ovarian cancer cell activation as well as in loss of clotting of cancer ascitic fluid.

Methods: The effect of aPC on ovarian cancer cell line (OVCAR-3) was tested in (i) adhesion and cell migration using wound healing assay and droplet test. (ii) cell signaling using protein phosphorylation- evaluated by Cyto-ELISA, (iii) cell cycle modification- assessed by flow cytometric DNA quantification, (iv) anti-coagulant activity- evaluated by the prolongation of partial thromboplastin time (aPTT) of a normal plasma in the presence or absence of aPC-treated ovarian cancer cells. In addition the soluble endothelial protein receptor (sEPCR), a trap for protein C, was quantified by ELISA in ascitic fluid of patients with ovarian cancer.

Results: In the ovarian cancer line OVCAR-3, aPC induces an increase in cell migration, that is inhibited by adding anti EPCR in the culture medium. The aPC activation of OVCAR cancer cells occurs via MEK-ERK and Rho-GTPase pathways evidenced by an increase in phosphorylation of threonine, and to a lesser extent in tyrosine phosphorylation, inducing cycle activation (G1 to S/G2). In addition, aPC-bound to OVCAR induces a 2 to 3-fold time prolongation of aPTT of normal plasma. It was also observed that in the peritoneal fluid of patients with ovarian cancer, s-EPCR concentration was $71 \pm 23 \text{ ng mL}^{-1}$ which was below the level observed in plasma of patients with ovarian cancer, that could explain the absence of fibrin deposit in peritoneal fluids.

Conclusion: Free aPC binds to membrane EPCR in the ovarian cancer cells, inducing cell migration via MEK-ERK and Rho-GTPase path-

way. This binding could also explain the absence of clotting of peritoneal fluids.

Disclosure of Interest: None declared.

PO118-MON

Estrogens upregulate TFPI-2 expression in MCF-7 cells probably by promoter demethylation

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Background: Tissue factor pathway inhibitor (TFPI)-2 is a matrix-associated protein inhibiting the activation of matrix metalloproteinases involved in tumor progression, invasion and metastasis. It is a tumor suppressor gene and methylation of its promoter is associated with reduced transcription of the gene. Estrogens have been shown to regulate the expression of DNA methyltransferases (DNMT) in estrogen receptor (ER) positive cells.

Aims: To investigate whether estrogens affect the expression of TFPI-2, and whether changes in methylation of the TFPI-2 promoter are involved.

Methods: MCF-7 (ER α positive) and MDA-MB-231 (ER α negative) breast cancer cells were treated with estrogens. TFPI-2 mRNA and protein levels were measured using qRT-PCR and Western blotting. The expression levels of DNMT1 and 3 were measured using qRT-PCR. The methylation status in the TFPI-2 promoter was evaluated using a bisulfite-sequencing PCR approach.

Results: The relative expression of TFPI-2 mRNA and protein was significantly upregulated after 6 h incubation with 17 β -estradiol (E2) or 17 α -ethynylestradiol (EE2) in MCF-7 cells, but not in MDA-MB-231 cells. This upregulation was reduced in the presence of fulvestrant, an ER antagonist. When the MCF-7 cells were treated with 5-aza-2'-deoxycytidine (decitabine), an inhibitor of DNA methyltransferases, the effect on TFPI-2 mRNA expression was similar to the effect of estrogens. Preliminary results suggest that estrogens and decitabine affect the expression of DNMT1.

Conclusion: In this study we found that estrogens significantly upregulated TFPI-2 expression levels in MCF-7 cells by a process involving ER α , possibly through regulation of DNA methyltransferases. Mapping of the methylation status of the TFPI-2 promoter before and after estrogen stimulation is in progress and will be presented.

Disclosure of Interest: None declared.

PO119-MON

Endothelial protein C receptor in breast cancer cells, co-expressed with multidrug resistance protein, inhibits fibrin formation in tumor micro environment

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Background: Activation of protein C is critical to the negative regulation of blood fibrin formation. In pathological conditions, endothelial protein C receptor (EPCR) is expressed in a large number of cancer cells, but the implication of EPCR/aPC in tumor-related inhibition of fibrin deposition in extra-cellular matrix is poorly detailed.

Aims: to analyze the role of EPCR in inhibition of fibrin deposition in breast cancer and the relation between EPCR and glycoprotein 1 (multidrug resistance protein, 1 p-gp) expression on tumour cells.

Methods: Expression of EPCR in several breast cancer cell lines (MDA-MB231^{P-Gp+}, MCF-7^{P-Gp+} and MCF-7^{P-Gp-}) was tested by

RT-PCR, and by immuno analysis such as dot blot, immunocytochemistry and flow cytometry. Tissue gene array and DNA sequencing was performed only in MDA-MB231^{P-Gp+}. Role of protein C in cell migration and cell reticulation was investigated by wound healing test and matrigel assay. Cell proliferation was measured by MTT test. Soluble EPCR (sEPCR) in peritoneal fluid was quantified by ELISA. Anti-coagulant activity of cancer cells incubated with active protein C (aPC) was analyzed by the prolongation of partial thromboplastin time (aPTT) of normal plasma.

Results: EPCR was detected in the great majority of breast cancer biopsies and in all cancer ascitic cell clusters and fluids. It was also expressed in breast cancer cell lines MDA-MB231^{P-Gp+} and MCF-7^{P-Gp+} but not by MCF-7^{P-Gp-}. Therefore, its expression correlated with chemoresistance and malignancy. aPC through EPCR binding, promotes cancer cell migration *in vitro* via PAR-1 activation and increases their survival. A 2 to 3-fold prolongation of aPTT of normal plasma was observed when aPC was incubated with cancer cells.

Conclusion: EPCR is co expressed with Pgp in chemoresistant breast cancer cells. aPC-EPCR binding induces cancer cell migration and by promoting unclottability of cancer cell microenvironment, participates in local matrix destabilization, thereby enhance cell dissemination.

Disclosure of Interest: None declared.

PO120-MON

Changes in coagulation in acute promyelocytic leukaemia

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Background: Despite improved treatment of acute promyelocytic leukaemia (APL) a significant minority of patients die from bleeding or thrombosis before or during early treatment.

Aims: To understand the mechanisms underlying thrombosis in APL.

Methods: Serial plasma and RNA samples were obtained from 32 newly diagnosed APL patients & stored at -80 °C. We measured PAI-1 (Trinitize, Diagnostica Stago), PF 1 + 2 (Dade Behring), and Tissue factor (TF) (Immubind TF, Invitech) by ELISA. We measured PAI1 and TF mRNA levels by RT PCR. Whole transcriptome profiling was performed on samples from patients with thrombotic events ($n = 5$), no thrombosis ($n = 60$) & healthy controls ($n = 5$). Genes with known roles in coagulation were identified and validated using quantitative PCR (qPCR) on these samples and APL cell lines (NB4 and PR9) before and after induction of *PML-RARA* and/or treatment with all-trans retinoic acid (ATRA).

Results: Of 12 candidate genes we validated two: TF (9 fold, $P = 0.0001$) and PAI-1 (2.8 fold $P = 0.032$) mRNA transcripts were expressed at significantly higher levels in APL patients compared to healthy controls and were modulated by ATRA treatment and induction of *PML-RARA* expression in the NB4 and PR9 cell lines.

Before treatment APL patients had markedly higher plasma levels of PF 1 + 2 compared to controls (1577 pmol L⁻¹ vs. 482 $P < 0.001$), TF (226 pg mL⁻¹ vs. 157 $P = 0.0118$) and PAI-1 (30.7 ng mL⁻¹ vs. 14.4). These levels did not change significantly during the first 5 days of ATRA treatment.

Conclusion: Patients with APL have evidence of activation of coagulation which may account for the increased incidence of thrombosis. Interestingly in this small ongoing study, plasma levels of TF and PAI-1 did not reflect mRNA expression levels.

Disclosure of Interest: None declared.

PO121-MON

Fibrinolytic activation in acute promyelocytic leukaemiaDillon R¹, Parmar K², Grimwade D¹ and Hunt B²¹Department of Medical and Molecular Genetics, King's College London; ²Department of Haematology, Guy's and St Thomas' Hospitals, London, UK**Background:** The outcome of acute promyelocytic leukaemia (APL) has improved with the use of ATRA, but a significant minority of patients still die from bleeding before and during treatment.**Aims:** To understand the mechanisms underlying haemorrhage in APL.**Methods:** We collected serial plasma and RNA samples from 32 newly diagnosed APL patients. We measured the expression of *ANXA2*, *S100A10* and *PLAUR* mRNA by real time PCR (RT PCR, TaqMan; Invitrogen). We measured D-Dimer, PAI-1 (both Trinitize, Diagnostica Stago) and Plasmin-Antiplasmin (PAP, Oxford Biosystems) by ELISA according to manufacturer's instructions. We measured PT (PT-Fib HS) and Clauss fibrinogen (FibC, Instrumentation Labs) using the ACL300R analyser.**Results:** *ANXA2* (3.5 fold $P = 0.002$), *S100A10* (7.6 fold $P < 0.001$), *PLAUR* (4.9 fold $P < 0.001$) mRNA transcripts were expressed at higher levels in APL samples compared with controls. Expression was significantly higher in those who experienced bleeding ($P = 0.021$, 0.032 and 0.022 respectively) and was rapidly downregulated after ATRA was initiated. These changes were confirmed in two APL cell lines (NB4 and PR9) and were *PML-RARA* dependent. Before ATRA treatment APL patients had increased plasma levels of PAP vs. controls (mean 3997 $\mu\text{g L}^{-1}$ vs. 461 $P < 0.001$). PAP > 2500 $\mu\text{g L}^{-1}$ was present in all patients with thrombosis or haemorrhage ($\chi^2 P = 0.046$). PAP levels rapidly normalised following ATRA treatment and inversely correlated with Clauss fibrinogen levels ($R^2 = -0.26 P = 0.0035$) and platelet counts ($R^2 = 0.53 P = 0.0022$) but positively with D-Dimer ($R^2 = 0.4 P = 0.0035$) levels. *ANXA2* ($R^2 = 0.33 P = 0.00253$) & *S100A10* ($R^2 = 0.40 P = 0.0116$) mRNA levels correlated strongly with PAP levels. Additionally *S100A10* mRNA levels correlated with PT ($R^2 = 0.48 P = 0.0039$).**Conclusion:** At least three cell surface molecules involved in fibrinolytic activation are upregulated by *PML-RARA* in APL and correlate with bleeding and plasma markers of fibrinolysis. This ongoing study suggests PAP may be a useful biomarker for bleeding or thrombosis in APL.**Disclosure of Interest:** None declared.

PO122-MON

Conditional inactivation of integrin αv subunit in vascular smooth muscle cells decreases thrombin generation in vessels and bloodMohamadi A¹, Li Z², Louis H¹, Bourhim M¹, Lacolley P¹ and Regnault V¹¹DCAC, U1116, Vandoeuvre-lès-Nancy; ²Vieillessement, Stress, Inflammation, UMR 8256, Paris, France**Background:** Integrins are transmembrane receptors that mediate cell-cell and cell-extracellular matrix interactions. Integrin $\alpha v\beta 3$ is expressed at high density in vascular smooth muscle cells (VSMCs) and weakly in platelets. It functions as a receptor for prothrombin in VSMCs whose phenotypic modulation plays a pivotal role in atherothrombosis.**Aims:** We investigated the role of integrin $\alpha v\beta 3$ in thrombin-generating capacity within the arterial wall or blood.**Methods:** Conditional transgenic mice inactivated for integrin αv subunit in VSMCs (αv^{CMLV}) were compared with their littermate controls.

Platelet reactivity was assessed by aggregation studies induced by ADP, collagen or thrombin agonists. Glycoprotein expressions at the surface of platelets were quantified by flow-cytometry. Thrombin generation was monitored using calibrated thrombography (CAT) in various conditions: in platelet rich (PRP) or poor (PPP) plasma but also in whole blood or in PPP to which an aortic ring has been added.

Results: As expected, platelet response to agonists and glycoprotein expressions were similar in αv^{CMLV} and control mice. Thrombin generation in whole blood, PRP and PPP was significantly decreased respectively by 20%, 10% and 14% in mutant mice compared with controls, indicating that this decrease is independent of blood cell surfaces. The addition of an aortic ring to PPP highly increased thrombin generation in controls while only a modest increase was observed in mutant mice.**Conclusion:** These findings highlight a hypocoagulable phenotype at the surface of aortic rings and in blood compartment of αv^{CMLV} mice. They demonstrate that VSMCs are able to generate thrombin involving $\alpha v\beta 3$ integrin and also suggest that this integrin could influence the conductance of coagulation factors such as tissue factor pathway inhibitor (TFPI) or antithrombin from the arterial wall to the blood compartment particularly in the presence of endothelium alterations**Disclosure of Interest:** None Declared.

PO123-MON

Oestrogens regulate TFPI expression through oestrogen response elements in TFPI 5' flanking regionAli HO^{1,2,3}, Stavik B^{1,2}, Myklebust CF^{1,2}, Dørum E^{1,2}, Iversen N⁴, Sandset PM^{1,2,3} and Skretting G^{1,2}¹Research Institute of Internal Medicine; ²Department of Haematology, Oslo University Hospital; ³Institute of Clinical Medicine, University of Oslo; ⁴Department of Medical Genetics, Oslo University Hospital**Background:** Oestrogens influence the pathology and development of hormone-sensitive breast cancers. Tissue factor pathway inhibitor (TFPI) has been shown to be associated with breast cancer pathogenesis. Recently, we found TFPI mRNA levels to be significantly reduced by oestrogens in a breast cancer cell line (MCF7), a process mediated through the oestrogen receptor alpha (ER α).**Aims:** To investigate the mechanism by which oestrogens may regulate TFPI at the transcription level.**Methods:** The TFPI 5'-flanking region contains three oestrogen responsive elements (ERE) half sites at positions -845, -769 and -50. Constructs containing the wild type ERE or mutated half sites of the TFPI 5'-flanking region were generated in a luciferase reporter gene vector and transiently co-transfected with an ER α expression vector into HEK293 cells and subsequently treated with oestrogens. A dual-luciferase reporter assay was used to assess luciferase activity. Electrophoresis mobility shift assay (EMSA) was used to study binding of nuclear factors to the various ERE half sites.**Results:** The luciferase activity was significantly downregulated with the wild type construct after oestrogen stimulation and this effect was abolished by mutation in either ERE half sites. EMSA analysis revealed direct and specific interaction of ER α with the ERE half sites at position -769 and -50 following stimulation with oestrogens. A weaker, but specific binding of the ERE half site at position -845 was also detected. Chromatin immunoprecipitation (ChIP) to verify binding of ER α is in progress.**Conclusion:** Results from the luciferase reporter assay and EMSA indicate that all three half sites may be involved in the oestrogenic regulation of TFPI expression. Our results suggest that oestrogens regulate TFPI transcription through ERE half sites in the TFPI 5' flanking region in ER α positive breast cancer cells.**Disclosure of Interest:** None declared.

PO124-MON

Monocytic leukemia cell lines possess enhanced procoagulant activity, the role of FLT3-ITD mutation

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Background: Monocytic leukemias with an unfavorable prognosis can be associated with FLT3 (fms-like tyrosine kinase receptor-3) internal tandem duplication (ITD). We utilized the wild-type THP-1, the heterozygous MOLM-13 and the homozygous ITD mutant MV4-11 cell lines to investigate their fibrin and thrombin generating capacity.

Aims: We evaluated the procoagulant activity (PCA) of three monocytic leukemia cell lines and hypothesized that the FLT3-ITD mutation status may be associated with altered PCA.

Methods: All cell lines were grown in RPMI-1640 medium, supplemented with 15% fetal bovine serum. Cell viability was studied by trypan blue exclusion and propidium iodide staining. One-stage clotting assay and thrombin generation test (TGT) were used to determine the procoagulant potential of cell lines as well as magnetically separated normal human monocytes. Tissue factor antigen (TF) and phosphatidyl-serine (PS) expression were examined by flow cytometry.

Results: There was no difference in PS expression (3–4%) of cell lines, but THP-1 cell line expressed the most TF followed by the MV4-11 and MOLM-13 cells (87%, 75% and 4.7% respectively). The PCA results were different in the cell lines resulting in 123 mU 5×10^{-6} THP-1 cells, 65 mU 5×10^{-6} MOLM-13 cells and 35 mU 5×10^{-6} MV4-11 cells. All three cell lines displayed a significantly faster thrombin generation (lagtime: 3.9–4.7 min) compared to normal human monocytes (lagtime: 14.9 ± 2.5 min). Factor VII deficient plasma and anti-tissue factor antibody pretreatment abolished the thrombin generating potential of all cell lines, but MOLM-13 cells also showed FXII-dependent residual thrombin generation. All cell lines generated microparticles (MPs) that showed the same thrombin generating tendency observed for intact cells.

Conclusion: TF is the primary contributor to the procoagulant potential of all cell lines that also generate MP with considerable PCA. FLT3-ITD mutational status may modulate the expressed procoagulants.

Disclosure of Interest: None declared.

PO125-MON

Thrombin drives pancreatic ductal adenocarcinoma growth and dissemination through mechanisms linked to fibrinogen and protease-activated receptor-1

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Background: Pancreatic ductal adenocarcinoma (PDAC), which accounts for greater than 85% of all cases of pancreatic cancer, is associated with extremely poor patient survival. High mortality is linked to the aggressive and invasive nature of the malignancy and poor efficacy of limited treatment options, which collectively highlight the need for novel treatment strategies. Notably, analyses of pancreatic cancer in patients and animal models have demonstrated that PDAC is associated with robust coagulation system activity driven in part by high tissue factor expression by the tumor cells (TF).

Aims: To determine the contribution of prothrombin, fibrinogen as well as tumor cell derived-TF and protease-activated receptor (PAR)-1 to PDAC growth and metastasis.

Methods: A novel tumor cell line (termed KPC2) was derived from mice in which PDAC tumorigenesis was induced by activation of two key pancreatic cancer alleles, *Kras*^{G12D} and *Trp53*^{R172H}. In transplant studies, tumor growth and experimental metastasis to the lung of KPC2 cells were evaluated in mice with genetically imposed deficits in coagulation system components. In addition, tumor growth and experimental metastasis were evaluated using KPC2 cells in which TF or PAR-1 expression was suppressed by shRNA knockdown.

Results: Primary tumor growth and experimental metastasis of KPC2 cells were significantly reduced in *fli^{low}* mice, which constitutively express 10% of normal prothrombin. Fibrinogen deficiency in mice significantly reduced tumor growth of KPC2 cells, but had little effect on experimental metastasis. PAR-1 knockout mice displayed similar growth and experimental metastasis of KPC2 cells. Notably, shRNA-mediated knockdown of either TF or PAR-1 in KPC2 cells resulted in significantly diminished tumor growth and experimental metastasis.

Conclusion: Our results suggest that thrombin can drive PDAC growth and dissemination through mechanisms linked to fibrinogen and tumor cell-derived PAR-1.

Disclosure of Interest: None declared.

PO126-MON

Microcirculation thrombosis in bone: a possible mechanism for bone pain

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Background: The blood supply of the bone and bone marrow are interconnected through a network of vessels. Arteries penetrating the bone flow into the marrow cavity while blood from marrow sinuses and veins leave the tissue *via* the bone.

Aims: The current study was designed to assess the presence of thrombosis in the small blood vessels of bone and bone marrow.

Methods: Bone biopsies of 40 patients were studied using specific staining to fibrin with martin scarlet blue (MSB) and by immune-staining to tissue factor (TF), TF pathway inhibitor (TFPI) and heparanase that is a pro-angiogenic and pro-coagulant protein. Biopsies included: ten cases of bone metastasis of carcinoma origin, ten cases of avascular necrosis (AVN) of the femur head and ten cases of osteoarthritis of the femur head. Ten cases of diffuse large cell lymphoma without bone or bone marrow involvement formed the control group.

Results: Vessel density was higher in the bone and bone marrow of metastasis, AVN and osteoarthritis biopsies compared to controls ($P < 0.001$). Thrombi were documented in the bone and bone marrow of metastasis (5/10), AVN (6/10) and osteoarthritis (6/10) biopsies and were absent in the controls. Staining of TF, TFPI and heparanase were more prominent, mainly in the blood vessels, in the biopsies of metastasis, AVN and osteoarthritis compared to controls.

Conclusion: The present study demonstrates for the first time thrombosis in the microcirculation of bone and bone marrow that can potentially contribute to patients' bone pain. Increased levels of TF and heparanase may partially account for the procoagulant and pro-angiogenic state. Intervention with anticoagulant drugs to prevent bone pain should be further evaluated.

Disclosure of Interest: None declared.

PO127-MON

Procoagulant effects of lung cancer chemotherapy through release of microparticles and cell-free DNALysov Z¹, Dwivedi DJ² and Liaw PC²¹Health Sciences; ²Medicine, McMaster University, Hamilton, Canada

Background: Lung cancer is the second leading type of cancer with venous thromboembolism (VTE) being the second leading cause of death among cancer patients. Studies have demonstrated increased levels of microparticles (MPs) and cell-free DNA (CFDNA) in lung cancer patients undergoing chemotherapy. However, the procoagulant effects of chemotherapy-induced MPs and CFDNA in lung cancer patients are unknown.

Aims: To study a) the effects of lung cancer chemotherapy-induced generation of MPs and CFDNA, and b) to determine the procoagulant effects of chemotherapy-induced MPs and CFDNA *in vitro* and *in vivo*.

Methods: MPs were isolated from monocytes, endothelial (HUVEC), or cancer (A549) cells. Tissue factor (TF) and phosphatidylserine (PS) expression was characterized and thrombin generation assays were used to determine MP procoagulant activity. Murine xenograft model of human lung carcinoma was used to quantify *in vivo* levels, and determine the procoagulant potential of TF-bearing MPs and CFDNA.

Results: Lung cancer chemotherapy increased TF levels and PS exposure on A549 cell MPs, but not HUVEC or monocyte MPs. Addition of A549 MPs to plasma enhanced thrombin generation. We observed elevated levels of CFDNA in plasma of chemotherapy-treated mice as well as increased levels of TF positive MPs in plasma of tumour-bearing mice treated with chemotherapy or saline. While both tumour-bearing and tumour-free mice treated with chemotherapy express a procoagulant phenotype, the effect was greater in tumour-bearing mice. Thrombin generation was not significantly attenuated by DNase treatment alone. However, removal of MPs alone or in combination with DNase significantly attenuated thrombin generation.

Conclusion: Our studies are the first to investigate the lung cancer chemotherapy-induced generation of procoagulant MPs and CFDNA. Our studies identify lung cancer MPs as the primary cell free initiator of coagulation. However, chemotherapy-induced CFDNA also contributes to the procoagulant effects of chemotherapy.

Disclosure of Interest: None declared.

PO128-MON

The Reed Sternberg cell coagulomeCesarman-Maus GN¹, Reichel J², Roshal M³, Braggio E⁴, Fonseca R⁴ and Cesarman E⁵¹Hematology, National Cancer Institute, Mexico, Mexico, Mexico; ²Pathology and Laboratory Medicine, Weill Cornell Medical College; ³Departments of Laboratory Medicine and Pathology, Memorial Sloan Kettering Cancer Center, NYC; ⁴Medicine, Mayo Clinic, Arizona; ⁵Departments of Pathology and Laboratory Medicine, Weill Cornell Medical College, NYC, USA

Background: Proteins involved in coagulation and fibrinolysis also play a role in inflammation, tumor progression, and the generation of tumor microenvironment. Classical Hodgkin's (cHL) is characterized by sparsely distributed Hodgkin and Reed-Sternberg (HRS) whilst the bulk of the tumor mass is made up of a reactive host background, complicating the acquisition of neoplastic DNA without extensive background contamination.

Aims: The objective was to study the gene expression of enzymes and receptors involved in coagulation and fibrinolysis (coagulome) of highly purified HRS cells. We previously showed that tissue factor gene (*F3*) is not expressed by T or B-cell non-Hodgkin's lymphomas, but was expressed in variable degrees in Hodgkin's lymphoma samples. These samples include reactive lymphoid tissue and scattered Hodgkin and Reed Sternberg (HRS) cells, which are the B-cell-derived neoplastic cells. Thus we aimed to determine if neoplastic HRS cells express or not *F3*.

Methods: We used flow-sorting of HRS and intra-tumor B cells with optimized low-input RNA sequencing and differential expression analysis of nine primary patient samples.

Results: As compared to tumor infiltrating B-cells, we found that TF is not expressed by HRS cells, nor are coagulation cascade enzymes, natural anticoagulants or inhibitors of fibrinolysis. Interestingly, we found overexpression of annexin 2A gene (*ANXA2*) by a mean of 8.8 fold (2 to 17.2) in HRS cells as compared to tumor infiltrating B-lymphocytes.

Conclusion: TF gene (*F3*) is not expressed by malignant HRS-cells, thus confirming that no B or T-cell derived tumor expresses TF. In contrast, of annexin family members, *ANXA2* is highly expressed in HRS cells. Annexin A2 protein is a receptor for plasminogen and tissue plasminogen activator, also known to be chemoattractant for monocytes which are typically present in Hodgkin's. The latter suggests that a role for annexin A2 in the generation of tumor microenvironment should be further studied.

Disclosure of Interest: None declared.

PO129-MON

Factor VII derived protein EGFP-EGF1 mediate drug delivery system for human tumor

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Background: Tumor stroma cells have been increasingly recognized to interact with tumor parenchyma cells and promote tumor growth. And therapeutics delivery to both parenchyma cells and stroma cells simultaneously might treat tumor more effectively.

Tissue factor (TF), an evolutionarily conserved protein, was extensively located in both tumor parenchyma cells and different types of stroma cells, with the potential as a favorable target for drug delivery to multiple cell types simultaneously. EGFP-EGF1 is a fusion protein derived from rat factor VII with special affinity for TF of rat derived C6 glioma cells, which might be utilized as a multi-targeting moiety to mediate drug delivery for human tumor.

Aims: We modified nanoparticles with EGFP-EGF1 protein and evaluated its multi-targeting possibility for human tumor.

Methods: Nanoparticles (NP) were developed via an emulsion/solvent evaporation technique and EGFP-EGF1 modified nanoparticles (ENP) was developed by maleimide-thiol coupling reaction. Human lung cancer cells A549 with high expression of TF was chosen as the tumor cell models. The targeting ability of ENP was verified by cell uptake experiment and *in vivo* imaging. The related multi-targeting mechanism was investigated by immunofluorescence staining. Finally, the pharmacodynamics of paclitaxel-loaded ENP (PTX-ENP) was also assessed on tumor-bearing mice.

Results: The modification of EGFP-EGF1 significantly improve nanoparticles uptake by A549 cells. *In vivo* imaging showed ENP achieved about 2.5 fold higher accumulation than NP. Results of immunofluorescence staining showed distribution of ENP in tumor parenchyma cell and tumor stroma including tumor-associated fibroblasts, tumor-associated macrophages and neo-vascular cells were more significantly than NP. Accordingly, ENP could destroy tumor more totally and

achieved more obvious tumor inhibitive effect than NP and other group.

Conclusion: The present study successfully established ENP as a multi-targeting drug delivery system for human tumor.

Disclosure of Interest: None declared.

PO130-MON

The role of M-components and immunoglobulins in patients with multiple myeloma - procoagulant and prognostic?

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Background: Venous thromboembolism (VTE) is a frequent complication in patients suffering from multiple myeloma (MM). The pathogenesis of the increased VTE-risk remains unclear. It is unknown whether the excessive production of M-components has a role in relation to this risk, i.e. whether a procoagulant activity can be linked to the M-components.

Aims: The aim was to investigate whether *in vitro* 'spiking' effect of serum-purified M-components compared to commercialized normal immunoglobulins modifies the thrombogenic profile in patients with MM, and if the isotypes of M-component are important for a potential procoagulant shift.

Methods: Peripheral blood was collected from 5 patients with MM (IgG or IgA isotypes) and matched with healthy donors (approved by the local ethics committee). M-components were isolated using affinity chromatography and verified through electrophoresis and immunofixation. Spiking was performed with purified M-components and normal immunoglobulins on plasma from both healthy donors and MM patients adopting a thrombin generation assay (calibrated automated thrombogram (CAT)).

Results: Thrombin generation spiking of plasma from both patients and controls with increasing amounts of purified M-component showed an augmented peak height and endogenous thrombin potential with shortened lag time and time to peak. A diminutive response was observed when spiking with normal immunoglobulins. Slight differences were detected between isotypes (heavy chain: α , γ ; light chain: κ , λ) spiking, but this may depend more on phenotypical differences for individual donors.

Conclusion: Spiking plasma with purified M-components clearly demonstrate a dose-dependent link between thrombin generation and the M-components. This indicates procoagulant properties of M-components, which, thus, may be important for the risk of VTE in patients with MM.

Disclosure of Interest: None declared.

PO131-MON

Hypoxia-independent tumor progression: activation of transcription factor HIF 1 via PAR1 and PAR2 receptors in breast cancer

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Background: Hypoxia has been pointed out as a crucial event for cancer progression, mainly acting through hypoxia inducible factors (HIF) activation. High levels of HIF-1 are indeed frequently observed in more aggressive tumors associated with worst prognosis. Interestingly, the expression of HIF-1 has been reported even in tumors unprovided with hypoxic areas, suggesting that oxygen deprivation is not the only way to induce HIF-1 in cancer.

Aims: The well-described contribution of inflammatory processes to tumor progression has led us to investigate if HIF-1 could be activated by PAR1 and PAR2 members of the proteinase-activated receptors family under normoxic conditions.

Methods: Human mammary carcinoma cell line MCF7 was treated with PAR1 and PAR2 agonist peptides and the expression and nuclear translocation of HIF was analyzed by confocal microscopy. Tumor cell migration was evaluated, under these conditions, using the boyden chamber. We used qRT-PCR to quantify *il8* and *il1b* mRNAs.

Results: Treatment of MCF-7 with PAR1 or PAR2 agonist peptides induces the expression and nuclear translocation of HIF-1 α subunit. Boyden chamber assays showed that factors released by MCF-7 cells, in a HIF-1-dependent manner, after treatment with AP-PAR1 and AP-PAR2 agonists induces tumor cells migration. These migration effects were correlated by qRT-PCR with the HIF-1-dependent transcriptional upregulation of *il1b* and *il8* genes after treatment of MCF7 cells with PAR2 agonist.

Conclusion: We conclude that pro-tumoral events induced by PAR1/2 receptors in MCF7 cells can be mediated by HIF-1, pointing out a possible new mechanism to breast cancer progression, independent of oxygen tensions in tumor tissues.

Disclosure of Interest: None declared.

PO132-MON

The photodynamic thrombi-specific nanoparticles starve solid lymphoma to death

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Background: As tissue factor(TF) is overexpressed on lymphoma, newly formed tumor capillary blood vessels distribute abundant TF because they lack tight junctions between endothelial cells.

Aims: In this study, EGFP-EGF1 protein-conjugated PEG-PLGA nanoparticle(ENP) was employed as a TF targeting vehicle, the hemaporphyrin monomethyl ether (HMME) was incorporated into it and the resulting vectors were evaluated as a TF targeted photosensitizer for activating the cascade and inducing tumors thrombosis.

Methods: Nanoparticles were developed via an emulsion/solvent evaporation technique. Rat brain microvascular endothelial cells(BMECs) after transfection of TF expressed were chosen as the tumor vessels endothelium cell models. The tumor models were the lymphoma-bearing mice that received i.v. administration of ENP-HMME or NP-HMME and illumination at the tumor site. The targeting ability of ENP-HMME was verified by cell uptake experiment and *in vivo* imaging. The related targeting mechanism including TF and reactive oxygen distribution was investigated by immunofluorescence staining,

Western-Blot and RT-PCR. The thrombus distribution was assessed by H&E staining.

Results: ENP-HMME uptake by BMECs was significantly more than NP-HMME. The TF expression of the BMECs increased 10 times after 30 s illumination in the ENP-HMME group. More than 4.25 times of nanoparticles gathered in the illumination site of ENP-HMME group than NP-HMME with illumination free group. Results of immunofluorescence staining showed the same results of nanoparticles accumulation with the distribution of TF and reactive oxygen both consistent with this performance. And H&E staining showed a marked increase of thrombi in the groups of ENP-HMME group.

Conclusion: As a TF targeted photosensitizer, ENP-HMME could not only enhance TF targeting ability but also selectively induce thrombosis in tumor vessels which might be a promising anti-tumor strategy.

Disclosure of Interest: None declared.

PO133-MON

Neutrophil extracellular traps mediate arterial and venous thrombus formation in a murine breast cancer model

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Background: Cancer patients are at increased risk of developing thromboembolic complications. Several mechanisms have been proposed to explain cancer-associated thrombosis including activation of host vascular cells. Recently it has been proposed that the release of neutrophil extracellular traps (NETs) contribute to the prothrombotic phenotype in cancer.

Aims: We have employed a murine mammary carcinoma model as a tool to evaluate the role of neutrophil extracellular traps in cancer-associated thrombosis.

Methods: The highly aggressive murine cell line, 4T1, was injected in the mammary fat pad of female balb mice. Tumor-bearing and control mice were subjected to either rose Bengal/laser-induced venous thrombosis model or FeCl₃-induced arterial thrombosis model.

Results: Tumor-bearing mice exhibited accelerated thrombus formation in the venous and arterial models as compared to tumor-free animals. Tumor-bearing mice exhibited increased plasma levels of free DNA and myeloperoxidase as well as significantly increased number of circulating neutrophils. Histological analysis of thrombus harvested from tumor-bearing animals exhibited a significant number of neutrophils. Remarkably, treatment with recombinant human DNase reversed the prothrombotic phenotype of tumor-bearing mice in both models.

Conclusion: NETs mediate both venous and arterial thrombus formation in tumor-bearing mice. These observations offer new potential targets for therapeutic intervention in cancer-associated thrombosis.

Disclosure of Interest: None declared.

Coagulation factor V, X, II, XIII – I

PO135-MON

Proteolytic inactivation of coagulation factor V by OmpT from enterohemorrhagic *Escherichia coli*

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Background: In response to bacterial infection, the innate immune response activates coagulation resulting in fibrin formation around the pathogen to limit its survival and growth. Enterohemorrhagic *Escherichia coli* (EHEC) may interfere with coagulation and fibrin barrier formation to colonize in the intestine. OmpT is an outer membrane protease of the Gram-negative bacteria *ompT* family of proteases that may impact coagulation.

Aims: To determine the effect of EHEC OmpT on Factor V (FV) structure and activity.

Methods: Wild type *E. coli* O157:H7 and an *ompT* deletion and over-expressing strain were used to determine the effect of cell and outer membrane vesicle (OMV) OmpT on FV activity in human plasma by microplate assay. Immunoblotting was used to correlate changes in FV activity with cleavage. The OmpT cleavage sites were determined with cleavage products of purified human FV incubated with the *ompT* over-expressing strain by automated Edman degradation.

Results: OmpT cleaved FV and inactivated its activity in human plasma. Immunoblotting demonstrated that FV cleavage correlated well with decreased activity. Wild type EHEC cells and OMVs inactivated FV in plasma by 60% after 2 h at room temperature and these effects were abolished with the *ompT* deletion mutant. Complementation of the *ompT* deletion mutant which results in *ompT* over-expression increased FV inactivation by EHEC cells and OMVs to 95% after 2 h at room temperature. OmpT inactivated FV by cleavage at K³⁴⁵ in the heavy chain and R¹⁰³⁴, Q¹²³⁶, Q¹²⁸¹, and Q¹³¹⁷ in the B domain resulting in four fragments of M_r 250 kDa, 150 kDa, 100 kDa and 45 kDa.

Conclusion: EHEC OmpT inactivation of FV would be expected to block fibrin barrier formation and induce an anticoagulant state thereby enhancing bacterial survival and growth. Inactivation of coagulation by OmpT may contribute to bleeding during severe EHEC infection which is associated with damage to intestinal mucosal cells resulting in hemorrhagic colitis, diarrhea and disseminated intravascular coagulation.

Disclosure of Interest: None declared.

PO136-MON

Combined FV AND FVIII deficiency (F5F8D) in a Chinese family with a novel missense mutation in MCFD2 gene

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Background: Mutations in LMAN1 or MCFD2 genes cause a rare autosomal recessive bleeding disorder, which was named combined FV and FVIII deficiency (F5F8D). Encoded proteins by MCFD2 and LMAN1 genes which may form a Ca^{2+} -dependent cargo receptor complex have been confirmed to participate in the transport of FV and FVIII from the endoplasmic reticulum (ER) to the Golgi. We made a diagnosis F5F8D by findings of decreased FV and FVIII levels in a Chinese family.

Aims: Try to study the molecular mechanism of the disease and observe the clinical features and treatment in this family.

Methods: We do a molecular genetic analysis in the family. All the exons of LMAN1 and MCFD2 genes were PCR amplified and sequenced.

Results: We found a novel missense mutations p.Asp81Ala in MCFD2 gene in a Chinese family. The patient was homozygous missense mutation for Asp81Ala in exon 3 of MCFD2 gene, while his father and one of his sisters were heterozygous. LMAN1 gene in this family was revealed no additional mutations.

Conclusion: The presence of consanguineous marriage in the family played an important role in the cause of this disease. The Asp81 missense mutations reported in MCFD2 to date was carried out from the many patients, like Asp81Tyr and Asp81Asn, while the mutations 'Asp81Ala' have not previously been reported. Therefore, It seems likely that the mutation in the Asp 81 is a hot region, The Asp81 residue replacement by Ala most likely disrupts the MCFD2-LMAN1 cargo receptor complex.

Disclosure of Interest: None declared.

PO137-MON

Successful abdominal operation without replacement therapy in a patient with combined FV AND FVIII deficiency due to novel homozygous mutation in LMAN1Wang A¹, Duan Q², Liu X¹ and Wu J¹*¹Department of Hematology, Anhui Provincial Hospital; Anhui Provincial Hemophilia Treatment Center, Hefei, China; ²Anhui Provincial Hospital, Hefei, China*

Background: Surgical operations in congenital coagulation factor deficiencies' patients will increase risk of perioperative bleeding if without adequate replacement therapy. After adequate replacement therapy, patients with hemophilias can have successful experience of surgical operation. Patient with congenital combined FV and FVIII deficiency (F5F8D) can also have successful percutaneous coronary intervention after replacement therapy. There are rare reports about successful surgical operations in patients with F5F8D without adequate replacement therapy.

Aims: We reported that a patient with combined FV and FVIII deficiency had successful abdominal operation without replacement therapy and analyzed the molecular mechanism of the disease for the patient.

Methods: Before the diagnosis of the combined FV and FVIII deficiency, a 35 years old female patient was admitted for abdominal operation due to hydrosalpinx and chocolate cyst of ovary. We do a molecular genetic analysis in the family. Peripheral blood DNA was

extracted. All the exons of LMAN1 and MCFD2 genes were PCR amplified and sequenced.

Results: Although with prolong activated partial thromboplastin time and prothrombin time, the patient underwent successful abdominal operation without replacement therapy. She tolerated well for abdominal operation therapy without increase risk of perioperative bleeding. Molecular analysis showed that the patient has a novel homozygous deletion mutation in exon 12 of LMAN1 (1456delGTG).

Conclusion: Our results suggest that a F5F8D patient with homozygous deletion mutation in exon 12 of LMAN1 (1456delGTG) can safely undergo abdominal operation therapy for hydrosalpinx and chocolate cyst of ovary without fresh frozen plasma and recombinant FVIII replacement therapy.

Disclosure of Interest: None declared.

PO138-MON

Engagement of the active site of thrombin by dabigatran or argatroban modulates its exosite-mediated interactions with fibrin and factor VAYeh C^{1,2}, Stafford A², Leslie B², Fredenburgh JC¹ and Weitz JI¹*¹Medicine, McMaster University, Hamilton, Canada;**²Thrombosis and Atherosclerosis Research Institute, McMaster University, Hamilton, Canada*

Background: Thrombin is a highly plastic molecule whose activity and specificity are regulated by exosites 1 and 2, positively-charged domains flanking the active site. Exosite binding by substrates and cofactors regulates activity by a) localizing thrombin, b) guiding substrates and inhibitors, and c) inducing allosteric changes at the active site.

Aims: Although exosite-to-active site allostery is well described, the impact of active site occupation on exosite function has not been well studied.

Methods: We examined the effects of the active site-directed inhibitors dabigatran, argatroban, and dansylarginine N-(3-ethyl-1,5-pentenediyl)amide (DAPA) on thrombin binding to (a) immobilized γ_A - or γ' -fibrin or factor Va using surface plasmon resonance (SPR), and (b) three-dimensional fibrin clots.

Results: Using SPR, dabigatran decreased thrombin bound to immobilized γ_A - and γ' -fibrin, and factor Va in a concentration-dependent manner with EC_{50} values of 184.6 ± 4.3 , 182.4 ± 15.0 , and 204.2 ± 17.0 nM, respectively. At saturation, dabigatran decreased thrombin binding to γ_A - and γ' -fibrin, and factor Va by 47.6 ± 0.4 , 28.4 ± 1.6 , and $37.9 \pm 3.2\%$, respectively. In contrast, argatroban and DAPA increased thrombin binding to γ_A - and γ' -fibrin, and FVa in a dose-dependent manner with EC_{50} values for argatroban of 62.4 ± 4.8 , 59.4 ± 5.1 , and 23.4 ± 8.8 nM, respectively, and for DAPA of 514.1 ± 24.0 , 515.9 ± 31.0 , and 565.1 ± 200 nM, respectively. The effects of the inhibitors determined by SPR were confirmed by measuring their effects on the binding of radiolabeled thrombin to three-dimensional fibrin clots.

Conclusion: These findings highlight the bidirectional nature of thrombin allostery and suggest that active site-directed thrombin inhibitors modulate exosite function. Therefore, active site-directed thrombin inhibitors appear to operate at multiple mechanistic levels, and our data suggest that dabigatran and argatroban may have divergent effects on thrombin-substrate interactions.

Disclosure of Interest: None declared.

PO139-MON

Factor VA in synergy with protein S enhances activated protein C binding to phospholipids

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Background: Activated factor V (FVa), a cofactor in the prothrombinase complex, can be proteolytically cleaved/inactivated by activated protein C (APC). The inactivation of FVa, which serves to inhibit thrombin generation, is greatly enhanced by protein S. The molecular mechanism(s) by which protein S enhances APC-mediated cleavage of FVa has not yet been fully established. However, it has been hypothesised that protein S enhances the association of APC to phospholipid surfaces. The role of FVa in the assembly of APC on phospholipid surfaces has not been defined.

Aims: To investigate how protein S and FVa (both individually and in combination), influence the binding of APC to phospholipids.

Methods: Binding of active-site-labelled (FITC) APC to phospholipid coated beads was evaluated using flow cytometry, in the presence and absence of protein S and/or FVa.

Results: The binding of 50 nM APC to phospholipids was enhanced by ~2-fold in the presence of protein S (100 nM). Whereas FVa (25 nM) alone did not show any enhancement upon APC binding to phospholipids, in combination with 100 nM protein S, it increased APC binding to phospholipids by 3 to 6-fold. Unlike protein S alone, however, the enhanced APC binding to phospholipids induced by FVa and protein S in combination appeared to be time dependent, causing a ~5.5-fold enhancement (by comparison to APC alone) after 1 min, and ~3.8-fold after 3 min. The enhancement by protein S alone was inhibited using anti-protein S antibodies. Interestingly, in the presence of FVa and protein S in combination, both anti-protein S and anti-FV antibodies were required to fully reverse the enhancement.

Conclusion: Protein S specifically augments the binding of APC to negatively-charged phospholipid surfaces. Moreover, FVa, in synergy with protein S, further enhances APC association to these surfaces. We hypothesise that through this mechanism, FVa increases the efficiency of its own APC-mediated inactivation in the presence of protein S.

Disclosure of Interest: None declared.

PO140-MON

Membrane cholesterol is a critical regulator of platelet-associated prothrombinase complex assembly

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Background: Activated platelets are a key site of prothrombinase complex assembly. They express distinct binding sites for factors Va and Xa yet do not bind factor Xa in the absence of factor Va, and they support rapid thrombin generation despite low phosphatidylserine expression. The origin of these unique properties is not known.

Aims: To define the role of cholesterol in the assembly and function of the platelet-associated prothrombinase complex.

Methods: Human platelets were isolated from consenting adults in accordance with the Declaration of Helsinki. To remove cholesterol, platelets (1×10^9 mL⁻¹) were incubated with an optimal concentration of methyl- β -cyclodextrin (5 mM; MBCD) for 5 min at 37 °C. Cholesterol depletion was monitored by the loss of ³H-cholesterol. Platelet activation with thrombin (5 U mL⁻¹) was assessed by P-selectin expression and factor Va release. Following activation, platelet-associated prothrombinase complex activity, the subpopulation of

platelets binding factor Xa, and the equilibrium binding of ¹²⁵I-factor Xa were determined.

Results: Treatment of washed human platelets with MBCD removed 40–50% of membrane cholesterol. MBCD-treated platelets were fully activated as indicated by P-selectin expression and factor Va release. Removal of platelet cholesterol resulted in a 35% decrease in prothrombin (1.4 μ M) activation rates. The K_M for prothrombin was unaffected, while the V_{max} of the reaction was decreased two-fold from 15.6 ± 2.2 to 8.5 ± 1.5 nM s⁻¹. Although cholesterol-depletion did not change the size of the platelet population that bound factor Xa, it did decrease the average number of factor Xa molecules bound per platelet from 1188 ± 158 to 402 ± 144 .

Conclusion: Cholesterol plays an essential role in regulating the assembly of the platelet-associated prothrombinase complex. This result challenges the paradigm that phosphatidylserine is the key lipid mediator of prothrombinase complex assembly.

Disclosure of Interest: None declared.

PO141-MON

Reference value and stability of factor V

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Background: Factor V is a glycoprotein that function in the common pathway of coagulation process as cofactor of factor Xa to activate prothrombin. Up to now factor V and factor VIII are recognized as labile coagulation factors. Generally monitoring of oral anticoagulant therapy is done by testing prothrombin time (PT) because three out of four vitamin K dependent factors (II, VII, IX, X) are assessed by this test. However, the result of PT is also influenced by the level of factor V and fibrinogen. As factor V is recognized as a labile factor, so the reduced level of factor V in stored plasma may prolonged PT.

Aims: The aims of this study is to determine the reference value of factor V activity in Indonesian population and to know the stability of factor V stored in cold condition.

Methods: Thirty five subjects consisted of healthy male and female, aged 18–65 year-old were recruited for determination of reference value of factor V activity. Blood samples were collected in sodium citrate in 9:1 ratio, and then were centrifuged at 2000 g for 10 min to obtain platelet poor plasma. Factor V activities were performed based on PT test utilizing factor V deficient plasma from Siemens and Thromborel S as a source of thromboplastin. The stability of factor V were studied using pooled plasma consisted 10 normal and abnormal plasma. Pooled plasma were aliquoting and divided into 4 groups. The first group was carried out immediately as based line. The second group were stored in 20° C, third group were stored in 4° C, and the fourth group were stored in -20° C. The factor V activities for each group were performed at 4 h, 12 h, and 24 h storage.

Results: The reference value of Factor V activities in Indonesian peoples were 80.4 to 160.3%.

Conclusion: Factor V was stable up to 4 h if stored at temperature of 20°C.

Disclosure of Interest: R. Dharma Grant/Research Support from: PT Sysmex Indonesia, R. Rudianto: None declared.

PO143-MON

Activated factor V inhibits the activation of factor IX by tissue factor/factor VIIa complex

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Background: In vitro experiments suggested that activated factor V (FVa) inhibits the tissue factor (TF)/FVIIa complex to activate FX.

Aims: Investigate if FVa inhibits FIX activation by TF/FVIIa complex and if this has an effect on thrombin generation (TG) in a large study population.

Methods: The effect of FVa on the FIXa generation triggered with TF/FVIIa or FXIa was measured in an in vitro reconstituted system using fluorescent substrates. The effect of FVa on TG was measured in a purified system triggered by TF (extrinsic) and by FIXa or FXIa (intrinsic). Plasma levels of coagulation factors FII, FV, FVII, FX, FVIII, FIX, FXI and FXII, as well as TG triggered with 1 pM or 5 pM TF, were determined in plasmas from 190 consecutive patients (selected for preoperative screening for bleeding disorders). Informed consent was obtained and study approval was obtained from the local medical ethical committee. Potential influence of FV on TG was analyzed by multiple regression analysis.

Results: In the reconstituted system, FVa inhibited the activation of FIX by TF/FVIIa in a dose-dependent manner, whereas FIX activation by FXIa was not affected. Furthermore, TF dependent TG in a purified system was dose-dependently inhibited by FVa. The intrinsic pathway, activated by FXIa or FIXa, was not inhibited by FVa. These findings were confirmed in patient plasmas, showing that the ETP and peak TG negatively correlated with FV levels (β between -0.31 and -0.45, $P < 0.001$), while the time to peak was positively associated with FV ($\beta=0.33$ for 5 pM TF, $\beta=0.28$ for 1 pM TF, $P < 0.001$).

Conclusion: In a purified system FVa inhibits the activation of FIX by TF/FVIIa complex. An inhibitory effect of FV on coagulation was supported by a negative association between FV levels and thrombin generation in plasmas from preoperative patients screened for bleeding disorders. Whether the FV-dependent inhibition of FIX activation occurs in vivo and the physiological relevance of this needs to be established.

Disclosure of Interest: None declared.

PO144-MON

The C-domains of pseudonaja textilis venom factor V contribute to cofactor stability but are dispensable for lipid-independent prothrombinase function

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Background: The essential cofactor Va (FVa; A1-A2-A3-C1-C2 domains) exclusively associates with the serine protease Xa (FXa) on a negatively charged phospholipid surface through its lipid-binding C-domains. Interestingly, FVa and FXa homologs from the venom of the Australian snake *Pseudonaja textilis* bypass this surface requirement to achieve complexation.

Aims: Here, we investigated whether the C-domains of venom-derived *P. textilis* factor V (ptFV) drive its lipid-independent cofactor function and, if so, can sustain this unique property in the setting of human FV.

Methods: To this end, we swapped the C-domains of constitutively active B-domainless human FV (hFV) and ptFV and expressed and purified the chimeric variants hFV-ptC and ptFV-hC. Using a purified prothrombinase assay with the lipid-independent substrate prethrombin-1, FV cofactor activity was examined with both the human as well as the venom-derived *P. textilis* FXa species in the presence or absence of saturating amounts of lipids.

Results: Surprisingly, ptFV-hC displayed full cofactor activity, irrespective of the availability of anionic membranes, while hFV-ptC functioned equivalent to hFV without gain-of-function in the absence of lipids. Consistent with this, the apparent affinity of the ptFV C-domains for phospholipid vesicles with 25–5% anionic content is 10–100-fold reduced compared to hFV. Interestingly, thermal stability analysis showed that ptFV is significantly more heat-stable than hFV. While the C-domain exchange adversely affected the thermal stability of ptFV, it did not affect that of hFV.

Conclusion: Overall, we conclude that the ptFV C-domains are of little relevance to its cofactor function and may have been evolutionary adapted to reinforce ptFV cofactor stability in favor over lipid membrane association. As such, our findings imply an alternative mode of macromolecular complex assembly in *P. textilis* venom that may have little or no bearing on the molecular dynamics that govern human prothrombinase assembly on phospholipids.

Disclosure of Interest: D. Verhoef: None declared, R. Camire Grant/Research Support from: Pfizer, Consultant for: Pfizer, P. Reitsma: None declared, M. Bos Grant/Research Support from: Bayer Hemophilia Awards.

PO145-MON

Assessing the role of complex N-linked glycans on the factor V light chain in endocytosis by megakaryocytes

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Background: Plasma-derived factor V is endocytosed by megakaryocytes via a two receptor system including low density lipoprotein receptor related protein-1 and an unidentified binding site. Endocytosis is mediated by the light chain region of factor V (1546–2196), and post-translational modifications are hypothesized to be involved in this process.

Aims: The goal of this study was to determine if complex N-linked glycans at Asn1675 and Asn2181 are involved in factor V endocytosis by megakaryocytes.

Methods: Native factor V was treated with glycosidases to selectively trim glycan chains. Deglycosylation was confirmed and quantified by tandem mass spectrometry. The effect of deglycosylation on factor V cofactor activity was assessed in a prothrombin activation assay. Endocytosis by megakaryocytes was subsequently determined by Western blotting.

Results: Mass spectrometry analyses indicated that treatment of factor V with neuraminidase and β -1,4-galactosidase resulted in the quantitative removal of all sialic acid residues and ~60% of the galactose residues from Asn1675 and Asn2181, destroying potential lectin binding sites. Prothrombinase assays indicated that factor Va cofactor activity was unaffected by sugar removal. Endocytosis of factor V (30 nM, 3 h) was not affected by deglycosylation as measured by quantitative Western blotting.

Conclusion: These combined observations indicate that trimming of complex N-linked glycans at Asn1675 and Asn2181 of the factor V light chain has no effect on factor V endocytosis by megakaryocytes or factor Va cofactor activity. These findings suggest no involvement for cellular lectins in factor V endocytosis.

Disclosure of Interest: None declared.

Coagulation factor VIII and IX – I

PO146-MON

N9-GP overestimation in one-stage clot method due to silica-mediated conversion to FIXa during contact activation

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Background The recovery of glycoPEGylated recombinant FIX (N9-GP) in one-stage (OS) FIX clot method using silica-based aPTT reagents is several-fold higher compared with that of plasma-derived FIX.

Aims: To identify the cause of N9-GP overestimation in the OS FIX clot method.

Methods: N9-GP and native FIX were incubated under conditions of, or mimicking, the contact activation phase of the OS FIX clot method using different aPTT reagents and deficient plasmas. Optionally, a purified enzyme replaced plasma and free PEG was added. Quantification of FIXa, FXIa and plasma kallikrein vs. time was done by enzymatic activity measurements on samples collected during the various incubations.

Results: Conversion of N9-GP, but not FIX, to FIXa occurred during contact activation in the presence of a silica-containing aPTT reagent, but not with two silica-free reagents. FXIa formation was similar with all three aPTT reagents. Compared with the rate of contact phase activation of N9-GP added to FIX-deficient plasma, there was approx. 50% reduction in FXI-deficient plasma, and undetectable activation in FXII- or prekallikrein-deficient plasma. Purified plasma kallikrein and FXIa could activate N9-GP in the absence of Ca²⁺, also requiring the presence of silica-containing aPTT reagent. This fits with the observed normal kallikrein generation in FXI-deficient plasma, but no FXIa formation in prekallikrein-deficient plasma. FXIIa on the other hand did not activate any form of FIX. Thus silica appeared crucial for the conversion of N9-GP to FIXa both in plasma during the contact activation phase and by purified enzymes (FXIa/kallikrein). The conversion was abolished by excess free PEG.

Conclusion: FXIa and kallikrein are responsible for the silica- and PEGylation-dependent, precocious contact phase activation of N9-GP in plasma, leading to overestimation in the OS FIX clot method. Conceivably, a silica surface mediates co-localisation of N9-GP (via the PEG moiety) and its activators.

Disclosure of Interest: P. Rosén: None declared, S. Rosén: None declared, M. Ezban Employee of: Novo Nordisk A/S, E. Persson Employee of: Novo Nordisk A/S.

PO147-MON

In vivo effect of recombinant FVIII (NOVOSEVEN®) and rFIX in a refined tail vein transection bleeding model in mice with haemophilia A and B

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Background: For in vivo effect studies, we have recently developed a refined tail vein transection (TVT) bleeding model in haemophilia A mice that has full anaesthetic coverage and avoids death/moribundity as endpoints. As previously shown, the new model is sensitive to replacement therapy with rFVIII.

Aims: To test the hypothesis that the new model would also be sensitive to rFVIIa bypass and rFIX therapies we examined the acute dose-

responses of rFVIIa and rFIX in haemophilia A and B mice, respectively.

Methods: The refined tail vein transection (TVT) bleeding model was applied with isoflurane anaesthetised F8-KO and F9-KO mice. Blood was collected in 37 °C saline and total blood loss quantified by haemoglobin.

Results: An inverse sigmoidal dose-response curve were fitted to blood loss data for rFVIIa with an ED₅₀ (mean and 95% CI) of 385 µg kg⁻¹ (252–590 µg kg⁻¹) and full normalisation, i.e. bleeding not different from wt controls, at 1080 µg kg⁻¹. For rFIX the ED₅₀ was 6.1 IU kg⁻¹ (3.3–11 IU kg⁻¹) with full normalisation at 44 IU kg⁻¹. Thus, in line with previously presented data on rFVIII, where the ED₅₀ was 1.1 IU kg⁻¹ (0.09–2.0 IU kg⁻¹) with full normalisation at 5 IU kg⁻¹, the model was also sensitive to pharmacological intervention with rFVIIa and rFIX. In addition to using the model acutely, it can also be employed in duration-of-effect experiments of rFVIIa, rFVIII, and rFIX and their potential analogues and derivatives.

Conclusion: The refined tail vein transection model exhibits sensitivity towards pharmacological intervention that is superior to the standard tail clip model and on par with tail vein transection using death/near-death as endpoint. Thereby, clinically relevant intervention with rFVIII and rFIX, but importantly also rFVIIa, can be evaluated in the refined model which eliminates the need for survival studies.

Disclosure of Interest: P. Johansen Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, M. Tranholm Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, C. Ley Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, T. Elm Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, J. Haaning Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, M. Ezban Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, T. Knudsen Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S.

PO148-MON

Early immunological events in AAV-F9 mediated ITI for hemophilia B mice with pathogenic fix inhibitors

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Background: Inhibitors for hemophilia B are difficult to eradicate using conventional ITI due to pathogenic anaphylactic responses and nephrotic syndrome. We have recently described a novel ITI therapy based on factor IX (FIX) expression in hepatocytes mediated by adeno-associated virus (AAV) F9 gene delivery in a inhibitor positive hemophilia B mouse model that develops pathogenic responses to FIX protein therapy.

Aims: The aims of the study were to determine early immunological events following AAV8-F9 ITI liver gene therapy.

Methods: Hemophilia B mice received six weekly exposures of 1 IU (40 IU kg⁻¹) FIX protein with antihistamine and PAF antagonist to prevent fatal anaphylaxis. Plasma was collected by tail bleed for baseline measurements. Mice were divided into control (PBS) or treated (AAV8-F9 1 × 10¹¹ g) groups and followed for 10, 14, and 21 days post AAV8-F9 gene delivery and plasma, spleen, and bone marrow cells were collected for analysis. aPPT and Bethesda assays were run on mouse plasma (Start4). FIX and anti-FIX IgG1 plasma levels were determined by ELISA and anti-FIX secreting B and plasma cells in splenocytes and bone marrow were determined by B cell ELISpot.

Results: Anti-FIX IgG1 levels were lowered as soon as day 10 following AAV8-F9 ITI, although we were able to detect plasma FIX by ELISA, maximal correction of hemostasis was on day 14. We detected anti-FIX IgG1 secreting B and plasma cells for all three time points (days 10, 14, and 21), even though there was a noticeable absence of inhibitors at the later time points (days 14 and 21) by IgG1 ELISA and Bethesda assay.

Conclusion: The rapid loss in plasma anti-FIX IgG1 levels observed in our study is most likely due to sequestration by FIX protein expressed from transduced hepatocytes followed by the suppression and not elimination of the antibody producing cells by regulatory T cells. These regulatory T cells most likely play a more prominent role in the long-term suppression and eventual elimination of FIX specific B and plasma cells.

Disclosure of Interest: None declared.

PO149-MON

Recombinant long-acting glycopegylated factor IX (nonacog beta pegol) in hemophilia B: assessment of target joints in the multinational randomized phase 3 clinical trial

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Background: The paradigmTM 2 phase 3 trial demonstrated overall median annualized bleeding rates of 2.93 in the 10 IU kg⁻¹ and 1.04 in the 40 IU kg⁻¹ once-weekly prophylaxis (PPX) arms. Estimated mean FIX trough activities during the trial were 8.5 IU dL⁻¹ in the 10 IU kg⁻¹ arm and 27.3 IU dL⁻¹ in the 40 IU kg⁻¹ arm.

Aims: This post-hoc sub-group analysis investigated the outcome of baseline-defined target joints in patients on PPX.

Methods: Patients were randomized to either 10 IU kg⁻¹ or 40 IU kg⁻¹ nonacog beta pegol once-weekly PPX for 52 weeks, or included in on-demand treatment for 26 weeks. Patients on PPX with target joints at baseline (defined as 3 or more bleeding episodes in a particular joint within a period of 6 months prior to trial) were included in this analysis. The trial was approved by independent ethics committees and patients provided written informed consent.

Results: In the 10 IU kg⁻¹ and 40 IU kg⁻¹ arm, respectively, 13/30 (43%) and 15/29 (52%) patients had at least 1 target joint at baseline, and of these 1/13 (7.7%) and 10/15 (67%) patients did not experience a target joint bleed during trial. 24 target joints were registered in each PPX arm at baseline, the majority being elbows (40%) and ankles (29%). 7/24 (29%) target joints in the 10 IU kg⁻¹ arm and 17/24 (71%) target joints in the 40 IU kg⁻¹ arm did not bleed during the trial.

By the recent ISTH definition (a target joint with ≤ 2 bleeds within 12 months is no longer considered a target joint), 58% of target joints in the 10 IU kg⁻¹ arm and 90% of target joints in the 40 IU kg⁻¹ arm had ≤ 2 bleeds during trial.

All target joint bleeds in the 40 IU kg⁻¹ arm were controlled with a single injection of 40 IU kg⁻¹ nonacog beta pegol.

Conclusion: According to the recent ISTH definition, 90% of the target joints in the 40 IU kg⁻¹ arm would no longer be considered target joints at end of trial. The results support the potential of nonacog beta pegol for routine PPX, prevention and treatment of bleeding episodes, and for reducing target joints bleeds in patients with hemophilia B.

Disclosure of Interest: C. Negrier Grant/Research Support from: Novo Nordisk, Baxter, Bayer, CSL Behring, LFB and Pfizer, Consultant for: Novo Nordisk, Baxter, Bayer, CSL Behring, LFB and Pfizer, Paid Instructor at: Novo Nordisk, Baxter, Bayer, CSL Behring, LFB and Pfizer, F. A. Karim: None declared, H. Hanabusa Paid Instructor at: Baxter Healthcare, Novo Nordisk, Bayer, Pfizer, Biogen Idec and KaketsuKen, Speaker Bureau of: Baxter Healthcare, Novo Nordisk, KaketsuKen and Biogen Idec, P. Collins Grant/Research Support from: CSL Behring, Consultant for: Novo

Nordisk, Baxter, Bayer, CSL Behring, Biogen Idec, Paid Instructor at: Novo Nordisk, Baxter, Bayer, CSL Behring, T. Colberg Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, B. Goldman Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, C. Walsh Consultant for: Baxter, Bayer, Novo Nordisk, CSL Bering, Pfizer, Hoffman La Roche, Speaker Bureau of: Novo Nordisk, Baxter.

PO150-MON

Assessment of recombinant factor IX-FC fusion protein (rFIXFc) clotting activity in plasma samples at canadian haemophilia treatment centres

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Background: A one-stage clotting assay using activated partial thromboplastin time (aPTT) is typically used to monitor factor IX (FIX) activity in patient plasma samples. New therapies are becoming available to haemophilia patients, such as a recombinant FIX-Fc fusion protein (rFIXFc). As these treatment options enter Canada, there is a need to understand monitoring capabilities and potential variability of these assays within local haemophilia treatment centres (HTCs).

Aims: The objective of this study was to assess the measurement of FIX activity in laboratories in Canada by distributing uniformly prepared frozen plasma samples containing various levels of rFIXFc.

Methods: FIX-immunodepleted plasma was spiked with rFIXFc at three different levels (0.80, 0.20, and 0.05 IU mL⁻¹) based on the manufacturer's labelled potency. Fourteen HTCs across Canada were enrolled in the study and each tested the samples using their own one-stage aPTT-based clotting assay and commercially available plasma standards.

Results: Of the fourteen participating HTCs, there were seven different aPTT reagent/instrument combinations which returned varying results for each spiked sample. For the 0.80 IU mL⁻¹ sample, results ranged from 0.41 to 0.95 IU mL⁻¹ (median = 0.72). Similarly, for the 0.20 IU mL⁻¹ sample there were a range of results from 0.11 to 0.35 IU mL⁻¹ reported (median = 0.21). Finally, results for the 0.05 IU mL⁻¹ sample varied between 0.03 and 0.12 IU mL⁻¹ (median = 0.06).

Conclusion: The wide ranges show the variability among HTCs in the measurement of FIX clotting activity of rFIXFc plasma samples. This variability could be due to differences in sample handling and analytical factors such as the aPTT reagent and coagulometer used. Previous studies have also revealed differences in FIX activity from other replacement products, and local centres need to be cognizant of this variability.

Disclosure of Interest: F. J. Geske Employee of: Precision BioLogic, A. Sadeghi-Khomami Employee of: Precision BioLogic, H. Chaudhry: None declared, M. Sholzberg: None declared, J. Teitel: None declared.

PO151-MON

A case of severe hemophilia B associated with a large insertion of SVA RETROTRANSPONSON in the coagulation factor IX gene

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Background: Hemophilia B (HB) is an X-linked recessive bleeding disorder caused by abnormalities of the coagulation factor IX gene (*F9*). The phenotypic severity of HB seems to be related to the position and type of the mutation. Insertion mutations in *F9* ranging from a few to more than 100 bp account for only a few percent of all causes of HB case.

Aims: To identify the causative gene mutation in a Japanese patient with severe hemophilia B, we investigated the abnormality in his *F9*.

Methods: The study was approved by the institutional committee for research ethics. After obtaining informed consent, we prepared genomic DNA of the patient from his peripheral leukocytes. We next performed direct DNA sequencing of the *F9*-specific PCR products and multiplex ligation-dependent probe amplification (MLPA) analysis for *F9*. We also performed a long-range PCR to identify the precise abnormality. In order to clarify complete sequence of the large insertion observed in the patient, we cloned a part of his *F9* containing the inserted region into pBluescript KS+ vector. Then, we prepared nested deletions of the insert using Exonuclease III and S1 nuclease, followed by sequence determination. We also performed an exontrap experiment to analyze abnormality in mRNA splicing events.

Results: In the patient sample, no *F9*-specific PCR product was amplified for exon 6, but MLPA showed a normal gene dose of exon 6. Long-range PCR revealed an approximately 2.5-kb SVA-F (SINE-VNTR-Alu subfamily F) element flanking by 15-bp duplications in the antisense orientation in exon 6, which causes a premature termination. Exontrap analysis showed only aberrant splice events of *F9* exons 5 and 6 in the SVA retrotransposition construct. No other causative mutation for HB was found in his *F9*. These data suggest that abnormal FIX mRNA may be degraded by nonsense-mediated mRNA decay leading to FIX deficiency in the patient.

Conclusion: We identified a large insertion of SVA retrotransposon leading to severe HB in a Japanese subject.

Disclosure of Interest: None declared.

PO152-MON

Tissue distribution of rIX-FP after intravenous application to rodents

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Background: rIX-FP, a recombinant fusion protein linking coagulation factor IX with albumin, is currently undergoing clinical trials in hemophilia B patients. Within a previously conducted QWBA (quantitative whole body autoradiography) study in rats following intravenous (i.v.) administration of radioactive-labelled rIX-FP, it was demonstrated that rIX-FP exhibits equal biodistribution but a prolonged tissue retention time compared to a marketed factor IX (FIX) product. However, rIX-FP tissue distribution on a protein and functionality level was not addressed in this study.

Aims: The aim of the present study was to evaluate the rIX-FP protein and activity levels in tissues following i.v. administration to rats and

FIX-deficient mice (hemophilia B mice), a commonly used model to mimic the situation in hemophilia B patients.

Methods: rIX-FP was i.v. administered to rats and hemophilia B mice at a dose of 2000 IU kg⁻¹. Thereafter, animals were euthanized at specific time points up to 72 h post dosing. Subsequently, plasma and various tissues were harvested and quantitatively analyzed for FIX antigen levels (rats and mice) using an ELISA assay as well as for FIX activity levels (mice only) using a chromogenic assay. For tissue samples, respective assays were performed in supernatants of tissue homogenates.

Results: Following i.v. administration to rats, rIX-FP distributed into all tissues analyzed (i.e. liver, kidney, skin and knee) with peak antigen levels reached between 1 and 7 h post dosing, thereby matching initial QWBA data. In hemophilia B mice, rIX-FP tissue distribution was comparable to rats using FIX antigen, but also FIX activity levels as readouts.

Conclusion: Our data confirm initial QWBA data showing that rIX-FP distributes into target tissues following administration. Importantly, it was demonstrated that rIX-FP available in tissues retains its functional activity and can thus facilitate its therapeutic activity at the site of potential injury.

Disclosure of Interest: S. Schenk Employee of: CSL Behring GmbH, B. Doerr Employee of: CSL Behring GmbH, F. May Employee of: CSL Behring GmbH, E. Herzog Employee of: CSL Behring GmbH, G. Dickneite Employee of: CSL Behring GmbH, M. Nolte Employee of: CSL Behring GmbH.

PO153-MON

The effects of a novel long-acting factor IX product (N9-GP) on wound healing

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Background: Hemophilia is a bleeding disorder caused by a lack of active coagulation protein (factor VIII in hemophilia A, factor IX in hemophilia B). To prevent uncontrolled bleeding, hemophilia B patients receive replacement factor IX (either plasma-derived or recombinant versions) as prophylaxis. Current standard therapies have a relatively short half-life and must be dosed approximately once every two to three days. A glycopegylated factor IX (N9-GP) in clinical development has demonstrated a longer circulation half-life and has shown to be well tolerated and efficacious when dosed weekly, which has promising implications for patient compliance and ease of use. Here, we evaluate N9-GP in a hemophilia mouse model of dermal wound healing.

Aims: To compare hemophilia B (FIX^{-/-}) mice treated with a single dose of N9-GP (285 or 470 IU/kg) to assess whether treatment corrects the wound healing defect observed in untreated FIX^{-/-} mice.

Methods: Excisional dermal wounds were placed on the backs of wild-type, untreated FIX^{-/-} and N9-GP-treated FIX^{-/-} mice using a 3-mm biopsy punch. The area of the wounds and number of healed mice were observed daily. At days 2, 4, 6, 8 and 10, some mice were sacrificed and wound samples were fixed for histological analyses.

Results: There was a dose-dependent decrease in wound area with N9-GP treatment. The healing times of the two N9-GP-treated groups were not different; both healed faster than untreated FIX^{-/-} mice but not as fast as wild-type mice. Paraffin-embedded wound samples were immunohistochemically stained to compare macrophage infiltration, iron deposition and angiogenesis between groups.

Conclusion: A single dose of N9-GP improved wound healing in hemophilic mice and decreased the iron accumulation and delayed macrophage influx associated with FIX^{-/-} wound healing. N9-GP also proved to be more effective than a single dose of rFIX, which did not affect the time to healing in this model.

Disclosure of Interest: E. Chappell Grant/Research Support from: Novo Nordisk A/S, M. Ezban Employee of: Novo Nordisk A/S, M.

Hoffman Grant/Research Support from: Novo Nordisk A/S, D. Monroe Grant/Research Support from: Novo Nordisk A/S.

PO154-MON

Inherited factor VIII and IX deficiency in patients reported to a tertiary care University hospital in India: a retrospective cohort analysis

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Background: Coagulation factor disorders are rare and most commonly inherited in an autosomal recessive pattern. These cases are largely asymptomatic and their adverse events get detected during bruises, cut, gum bleeds and menorrhagia. Early diagnosis ensures the patient with proper care and treatment.

Aims: To evaluate the clinico-pathological, therapeutic and economic outcomes with coagulation Factor VIII and IX disorders (F-VIII&IXD) [ICD-10 D66, D67].

Methods: A retrospective cohort analysis on patients admitted during 2012-13 was carried out with F-VIII&IXD at KMC hospital Manipal, West coastal region of Southern India.

Results: Seventy-eight patients diagnosed with F-VIII&IXD. The median age of onset of bleeding was <1 year in which all were males. 67% of patients were found to have a strong family history of which 58% were offspring from consanguineous marriage. Religion-wise 60% were Hindus, 36% Muslims and others were 5%. Bleeding complications (left knee hemarthrosis) were the most predominant seen in 71% of the patients. Factor assays in 40% showed severe hemophilia presenting with 65% Grade III, 23%>Grade II and 13% Grade I anemia, moderate hemophiliacs were 40% with 58% Grade III, 26%>Grade II and 16% Grade I anemia and 20% with mild severity who had 81% Grade III, 19% Grade II with no Grade I anemia. Management was in accordance to Agency for Healthcare Research and Quality guidelines. F-VIII&IX concentrates; Tranexamic acid and Fresh Frozen plasma/whole blood/Cryoprecipitate were used for symptomatic, maintenance and supportive therapy respectively. Cost of therapy per admission per patient was calculated to be ≈US\$350.

Conclusion: F-VIII&IXD are inherited in both sexes were it expresses in males predominantly while offsprings from consanguineous marriage, common in India are at greater risk. Patients who can afford standard treatment and follow-up show better symptom free period as compared to those who couldn't, is an alarm for administrators and policymakers.

Disclosure of Interest: None declared.

PO155-MON

Protein S, a new agent in adjunct therapy of hemophilia B

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Background: Current treatment of Hemophilia B consists of infusion of factor IX (FIX) concentrates to substitute for deficient FIX, i.e., replacement therapy. Yet, replacement is only temporary, as infused FIX is cleared rapidly from a patient's plasma. We found that Protein S (PS) inhibits FIX, and, importantly, anti-PS antibody increased FIX activity in Hemophilia B plasma, implying that blocking PS activity may achieve longer lasting replacement therapy.

Aims: To assess the effectiveness of anti-PS antibody in reducing clotting time.

Methods: Clotting, thrombin generation, isolated factor assays.

Results: We used a modified aPTT assay (clotting initiated with FIX) with FIX-deficient plasma and varied the concentrations of added FIX and anti-PS antibody. The aPTT clotting times in the presence and absence of anti-PS antibody were measured for 2.5 (51 sec +Ab; 69 sec -Ab), 5 (43 sec +Ab; 59 s -Ab), 10 (38 sec +Ab; 51 sec -Ab), and 20 nM FIXa (33 sec +Ab; 40 sec -Ab). Results showed that in FIX-deficient plasma, an anti-PS antibody would make added FIXa ~3 fold more active. We also performed a thrombin generation assay with the same FIX-deficient plasma in the presence of 1, 2.5 and 5 nM FIXa and measured peak thrombin formation in the presence of anti-PS antibody. Both the thrombin generation assay and the clotting assay gave similar results, i.e., addition of neutralizing anti-PS antibody made FIX ~3 fold more active. In wild type mice we have found that PS inhibited thrombin generation. Work is underway to assess hemostasis in Hemophilic B mice receiving a low dose of FIX and PS antibodies. Improved hemostasis in this mouse model would move anti-PS antibody to the forefront as a possible adjunct in Hemophilia therapy.

Conclusion: Our findings suggest that administration of anti-PS antibodies to hemophilia B patients may achieve the goal of longer lasting replacement therapy. Antibody blocking PS activity towards FIXa is the most straightforward approach and one most likely to succeed.

Disclosure of Interest: None declared.

PO156-MON

In vivo efficacy of human recombinant factor IX produced by the human hepatoma cell line HUH-7

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Background: Post-translational modifications (PTM) of the CHO-cell-derived-recombinant human factor IX (FIX) currently used for the treatment of hemophilia B (HB) are different from plasma derived FIX. Our previous results described a rFIX (HIX) produced by Huh-7 cells having better PTM profile than rFIX produced by CHO cells.

Aims: The study consisted to verify the improved PTM effect of HIX *in vivo*. We hypothesized that the efficacy of HIX previously identified *in vitro* might be verified after injection in HB mice.

Methods: HIX has been produced in a bioreactor and then purified from supernatants. *In vitro* activation and activity were evaluated, measured by thrombin generation tests (TGT) and compared to commercial molecules, Benefix[®], Mononine[®]. The three molecules were then administrated (i.v.) to FIX-knockout mice (at the dosage of 10 µg FIX/20 mg body weight) and two min after injection, blood samples were collected and subjected to human FIX-specific-ELISA and TGT.

Results: The clotting function of HIX, activation courses of HIX by FXIa and FVIIa-TF complex or by FXIa appear normal as did activation of Benefix[®], Mononine[®] and TG constants of each FIX were equivalent. After injection to HB mice, circulating HIX did not present any significant difference in term of antigen value with Benefix[®]. Intriguingly, TGT were clearly exhibiting a better velocity and thrombin peak for HIX than Benefix[®] and Mononine[®]. These data suggested that HIX may improve *in vivo* coagulant efficacy in comparison with the two commercial FIX injected at the same dose.

Conclusion: The study shows that HuH-7-derived-rFIX has better *in vivo* haemostatic activity in hemophilia B mice compared to the reference rFIX molecule despite similar *in vivo* recovery rates, suggesting that HuH-7 cells could represent an effective cellular system for production of rFIX.

Disclosure of Interest: None declared.

PO157-MON

Incorporation of mutations at E410 of factor IX improves clotting activity in vitro and in vivo

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Background: Replacement therapy in severe Hemophilia B (HB) is very effective but is limited by FIX concentrates injections frequency and cost issues. Production of a recombinant FIX (rFIX) with enhanced clotting activity and prolonged half-life is one of the current challenges for HB treatment. We previously reported four modified rFIX mutants with a substitution at glutamate E410 (E410H, E410A, E410L and E410N) presenting a 3 to 5 higher specific activity in-vitro.

Aims: The study consisted to understand the causes contributing to the increased clotting activity of FIXE410 by determining several functional parameters in vitro and in vivo.

Methods: Recombinant FIX molecules were produced by the Huh-7 cell line. Interaction of FIXE410 mutants with FX and FVIIIa, were compared to FIXwt using FXa generation assays. HB mice were injected with FIX molecules (10 µg FIX/20 mg body weight) for kinetic parameter determination. Washed platelet suspensions were prepared from pooled whole blood of five HB mice for ex-vivo FIX binding assays.

Results: In the presence of FVIIIa, the apparent Kd of FIXE410 varying from 1.55 to 2.35 nM were lower than the Kd of FIXwt 3.50 nM. The three variants FIXE410H, FIXE410L and FIXE410A exhibited 2.2-fold higher affinity for FVIIIa while FIXE410N had 1.5-fold higher affinity compared to FIXwt. Finally, the Vmax of the FIXE410H was increased by 3.9 fold in comparison with the FIXwt. Two min after infusion to HB mice, the concentrations of FIXwt in mice plasma were 4-fold higher than FIXE410H. However, FIXE410H induced the highest clotting activity and thrombin generating capacity in vivo. We have demonstrated a rapid and increased binding of FIXE410H to mice platelets compared to FIXwt explaining the decrease in the in-vivo recovery.

Conclusion: We have engineered and characterized four improved FIX proteins with enhanced in vitro and in vivo activity. These results suggest that these new molecules could optimize protein replacement therapy for HB treatment.

Disclosure of interest: None declared.

PO158-MON

The sleeping-beauty transposon technology for the generation of cellular and animal models of haemophilia B and the assessment of RNA-based therapeutic approach

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Background: Intervention at the mRNA level is emerging as therapeutic strategy for genetic disorders, which may overcome some limitations of gene replacement therapy. Variants of the spliceosomal U1snRNAs have been exploited to correct splicing mutations, but mainly in minigene assays. This highlights the need for appropriate mutation-specific cellular and animal models.

Aims: To exploit the Sleeping Beauty Transposon (SB100X) for the development of cellular/mouse models of Haemophilia B (HB) caused by the FIXex5-2C splicing variant, in order to assess the U1-mediated correction in a genomic context.

Methods: Co-transfection of Hek293 with plasmids (p) for the transposon/splicing competent-human FIX (SChFIX) variants and the SB100X, and measurement of FIX mRNA (RT-PCR) and protein (ELISA). Hydrodynamic injection of pSChFIX with the pSB100X in wild-type C57BL/6J mice, and evaluation of hFIX expression by species-specific assays. Delivery of the therapeutic U1sh9 via adeno-associated virus (AAV) vectors-mediated gene transfer.

Results: We created stable clones for the FIXex5-2C variant displaying the FIX aberrant splicing pattern (exon 5 skipping), which was rescued (from undetectable to ~45% of correct transcripts) by the U1sh9, and resulted in significant increase of secreted FIX levels (from ~3 to ~45 ng mL⁻¹, $P < 0.05$). Mice injected with the hFIX cDNA and pSB100X expressed high and stable protein levels (100 ng mL⁻¹–2.5 µg mL⁻¹), while low FIX expression was obtained with the SChFIX mutant cassette (<10 ng mL⁻¹ 1 month post-injection). Rescue of the FIXex5-2C mutant *in vivo* is ongoing, with evidence of efficacy of the AAV-mediated delivery of the U1sh9.

Conclusion: The SB-Technology represents a useful platform for the creation of mutation-specific HB models, which will be exploited in *F9 KO* mice to assess the impact of the U1snRNA-mediated FIX rescue on coagulation efficiency and hemorrhagic phenotype. The approach could be extended to other molecular defects to investigate alternative therapeutic options.

Disclosure of Interest: None declared.

PO159-MON

Comparison of factor IX glycosylation in different age groups

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Background: Factor IX (FIX) is a critical coagulation factor, with deficiencies resulting in hemophilia B, a severe bleeding disorder. In children FIX levels are significantly reduced vs. adults. Human FIX contains 2 potential N-linked glycosylation sites which are fully occupied in the adult molecule. Protein glycosylation is known to affect pharmacodynamics and function, and previous research has shown that glycosylation of some coagulation factors can differ in newborns vs. adults.

Aims: To compare the N-linked glycosylation profiles of FIX in adults and newborns.

Methods: Adult pooled plasma was purchased commercially. Newborn plasma was obtained from umbilical cords after delivery of healthy full-term babies and pooled (≥ 28 donors). FIX from adult or newborn plasmas was partially purified by immuno-affinity chromatography. FIX was deglycosylated with N-glycosidase F (PNGaseF) and apparent molecular weight (MW) determined by SDS-PAGE. To evaluate sialic acid content, plasmas were treated with and without neuraminidase and subjected to native PAGE with immunoblotting for FIX detection.

Results: Adult and newborn FIX had similar apparent MW on SDS-PAGE (69.1 ± 2.1 kDa and 68.7 ± 2.5 kDa respectively, $n = 3$). Treatment with PNGaseF caused a shift to a similarly lower apparent MW for both molecules (57.5 ± 2.0 kDa and 58.0 ± 2.0 kDa, $n = 3$). Native PAGE assessment indicated no major difference in migration between adult and newborn FIX. After treatment with neuraminidase migration decreased but again there was no apparent difference between adult and newborn proteins.

Conclusion: There is no observed macroheterogeneity in total N-glycan content between adult and newborn FIX and no clear difference in sialic acid content. Therefore, the trend towards altered glycosylation in adult vs. newborn coagulation factors is not demonstrated in FIX, which may indicate a critical role for these structures throughout life.

To our knowledge this is the first study of FIX glycosylation in newborns.

Disclosure of Interest: None declared.

PO160-MON

Investigations into anticoagulant resistance and enhanced activation as putative molecular mechanisms of the hyperactivity of factor IX Padua

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Background: The naturally occurring hyperactive Factor IX (FIX) Padua, due to the Arg338Leu substitution, provides appealing possibilities to improve therapeutics for hemophilia B (HB). The eight-fold increase in specific activity of this variant compared to wild type (WT) allows for a decrease in the therapeutic dose while simultaneously maintaining hemostatic efficacy. Indeed, there are ongoing and planned clinical gene-therapy trials for HB utilizing this approach. Despite these exciting therapeutic potentials, the molecular mechanism responsible for the hyperactivity remains unknown.

Aims: To determine the mechanism of FIX Padua hyperactivity.

Methods: Recombinant factor expression, clotting, thrombin generation.

Results: In a complete twenty amino acid scan at position 338, we observe that FIX-Padua is the most active variant, while FIX-WT is one of the least functional. Moreover, more than two-thirds of substitutions result in FIX variants with at least equal activity compared to WT. We see no evidence of spontaneous activation of FIX-Padua during recombinant expression and purification and *in vitro* kinetics of FIX activation by activated FXIa are similar between FIX-Padua and WT. We also observe the same increase in both clotting activity and thrombin generation of the Padua variant compared to FIX-WT when using either the zymogen or activated enzyme. Finally, the hyperactivity of FIX-Padua is not affected by decreasing the Protein S concentration.

Conclusion: The ability of the majority of substitutions to increase FIX activity suggests that the hyperactivity is more likely due to a disruption of a molecular interaction, such as would occur if FIX-Padua was resistant to endogenous anticoagulants. However, Protein S does not seem to be involved. Most importantly, our data demonstrates that the hyperactivity of FIX Padua is also *not* due to differences in FIX activation. These initial results support the safety of FIX Padua as it moves forward in clinical trials.

Disclosure of Interest: None declared.

PO161-MON

Variety of F9 mutation identified in 43 Japanese hemophilia B patients

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Background: Hemophilia B is X-linked recessive inheritance bleeding disorder caused by coagulation factor IX (FIX) gene (*F9*) mutations resulting in an absence or reduced activity of FIX. The *F9* is located at Xq27.1 with 8 exons and encodes a 2.8 kb mRNA. In 1,095 kinds of mutation of Factor IX Variant Database (<http://www.factorix.org>), a point mutation was most common with 72.9%.

Aims: We performed *F9* gene analysis to examine the relationship between genotype and phenotype.

Methods: Informed consent was obtained from the patients who participated in the study. The study was approved by the Ethics Committee of Tokyo Medical University, Tokyo, Japan. Purified PCR templates from genomic DNA were analyzed by direct sequencing and MLPA.

Results: A total of 43 patients with hemophilia B were included in this study. Twenty-eight patients were classified as severe hemophilia B (FIX activity <1%). Among them, we observed 14 missense (50%), 8 nonsense (29%), 1 splice site (4%) mutations and 5 small deletions (18%). In 15 moderate and mild patients, we observed 11 missense (73%), 2 nonsense (13%) and 2 splice site (13%) mutations. Thirty-three distinct *F9* mutations were identified, including 20 missense (61%), 7 nonsense (21%), 3 splice site (9%) mutations and 3 small deletions (9%). Duplication and large deletion were not detected by MLPA. Novel 9 mutations, included 7 missense (p.Gly50Asp, p.Ala74Pro, p.Asn80Thr, p.Gln96Lys, p.Pro239Arg, p.Leu346Pro, p.Ala337Leu), 1 donor splice site (c.838 + 3A>C) and 1 frameshift (c.1318 delA) mutations, were identified. Seven mutation hot spots (p.Arg43Gln, c.159-160 delAG, p.Ala74Pro, p.Arg294*, p.Arg298*, p.Arg384*, p.Gly413Glu) were identified. Therefore the p.Ala74Pro was detected only in this study, the findings suggested that the novel mutation was a founder-effect in Japanese.

Conclusion: As the first inclusive report, we demonstrated that a variety of mutations in *F9* were causes of Japanese patients with hemophilia B.

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PO162-MON

Location of two novellargegene deletions in Chinese haemophilia B patients

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Background: Haemophilia B (HB) is a rare X-linked recessive bleeding disorder by mutations in the coagulation factor IX (FIX) gene. Large gene deletions occur in a minority of hemophilia B patients. Hitherto, few partial gene deletions have had breakpoints defined.

Aims: The aim of the study was to identify the characterization of the causative mutations in the FIX gene in HB patients from central China and to localize the breakpoints of the partial FIX gene deletions.

Methods: For the propositi and suspicious carriers, all regions of FIX gene, including all exons and the flanking sequences, were amplified

by Polymerase Chain Reaction. Screening of the factor IX gene was performed by direct sequencing. Using a 5' primer for the fragment of exon before the exon that couldn't be amplified (Exon forward) and a 3' primer for the fragment of exon after it (Exon backward), large gene deletion of the suspicious subject was approved in comparison to the normal control by long distance-PCR and agarose gel electrophoresis. Several pair primers were designed to cover all areas from Exon forward to the putative breakpoint and from the putative breakpoint to Exon backward, respectively, which were used to localize the breakpoint.

Results: Twenty different FIX gene mutations were identified in the 20 HB patients, 3 had large gene deletions (1 total and 2 partial), 2 small deletions, and 15 single nucleotide substitutions. The two partial gene deletions are analyzed, they are g.10413_11363del953 and g.12163_23369del11207, the coagulation activity of FIX (FIX:C) are 6% and 0.7%, respectively. We also discovered a homozygous missense mutation c.571C>T, with amino acid changing Arg¹⁹¹ into Cys in the exon 6 of a 3-year old mild HB (FIX:C -19.5%) subject's FIX gene.

Conclusion: The FIX mutations were heterogeneous and the missense mutations were the most prevalent gene defects. The location of two novel large gene deletions were defined.

Disclosure of Interest: None declared.

PO163-MON

Quantitation and visualisation of nanosize protein aggregates in rFIX and pdFIX concentrates

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Background: Non-native protein aggregation is a prevalent problem occurring in many biopharmaceutical manufacturing processes. Changes in protein structure can expose internal hydrophobic regions of the polypeptide chain, forming larger assemblies of misfolded proteins. A discussion is currently ongoing between regulators, industry and academia to determine whether sub-visible particles could pose a significant risk (Den Engelsman et al. Pharm Res 2011).

Aims: As the presence of aggregates in biopharmaceutical formulations remains a quality and safety concern we analyzed the amount of sub-visible protein particles in rFIX and pdFIX concentrates.

Methods: Subvisible particles < 400 nm were examined in 1 rFIX product (Benefix) and 7 pdFIX products (Alphanine, Berinine, Factor IX Biotest, Immunine, Haemonine, Mononine, Octanine) using a Nano-Sight Nanoparticle tracking analysis system. The technique combines laser light scattering microscopy with a charge-coupled device camera, which enables the visualization and recording of protein aggregates in solution, relating the degree of movement under Brownian motion to the sphere equivalent hydrodynamic diameter particle size. The FIX concentrates were diluted with nanoparticle-free water (negative control). To avoid potential external contamination preparation of samples was performed using a laminar flow hood.

Results: In Benefix only very few, small (<250 nm) sub-visible particles were found. pdFIX concentrates contained a lot more particles with different sizes. Many small particles and a few particles up to 480 nm were found in Berinine and Haemonine. Immunine contained small particles, but also particles with average sizes of 350 and 600 nm.

Conclusion: Hemophilia patients are treated for lifetime, and therefore products for their treatments need to be controlled to the greatest possible extent.

Ex vivo studies should be initiated to measure the amount of injected misfolded proteins / protein particles and the influence of these product impurities in vivo.

Disclosure of Interest: None declared.

PO164-MON

Inhibitor development in relation to treatment duration in severe hemophilia A in previously untreated patients: results from the sipet trial

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Background: The development of neutralising antibodies (inhibitors) to FVIII is a serious complication of haemophilia treatment. The risk is very high in the early phase of treatment (~25%), and declines sharply afterwards (~0.5% per year). It is unclear when patients can be considered to have overcome the early high-risk phase, which is important for the design of trials of new coagulation products.

Aims: To correlate inhibitor development with the duration of treatment in previously untreated patients (PUPs) with severe hemophilia A.

Methods: SIPPET is an ongoing randomized trial contrasting two classes of FVIII concentrates produced from human plasma or by recombinant DNA technologies, in previously untreated or minimally treated (<5 EDs) children with severe haemophilia, who are 1:1 randomized to one of the two classes of concentrates. Patients are followed up until inhibitor development or until 50 EDs or 3 years from enrolment, whichever occurs first. Total sample size is 300. The current analysis was performed at the time of the planned interim analysis, after 204 patients had been randomized. An outcome event is defined as an inhibitor level ≥ 0.4 BU mL⁻¹. High responding inhibitor are defined by peak levels >5 BU mL⁻¹. Positive inhibitor tests had to be confirmed twice centrally. Analyses included time-to-event life-table analysis and cross tabulation. All results presented here are for the two treatment arms combined.

Results: Two hundred and four patients were included, aged 0–72 months (median 20 months), from 13 centres in four continents, followed for a mean of 26 ED. 54 patients developed an inhibitor, of

which 32 were high-titred. Cumulative incidence of all inhibitors was 32%. Inhibitors developed at a mean of 9.5 ED. All inhibitors developed before 38 ED, and over 90% before 20 ED. All high-titre inhibitors had developed by 20 ED.

Conclusion: Results from this randomised and prospective study show that neutralising antibodies in previously untreated patients with severe haemophilia A develop before 20–40 exposure days.

Disclosure of Interest: F. Peyvandi Grant/Research Support from: Alexion, Bayer, Biotest, Kedrion Biopharma and Novo Nordisk, Consultant for: Grifols, Kedrion Biopharma, LFB and Octapharma, Speaker Bureau of: Alexion, Baxter, Bayer, Biotest, CSL Behring, Grifols and Novo Nordisk, P. M. Mannucci Speaker Bureau of: Bayer, Grifols, Kedrion Biopharma and Novo Nordisk, I. Garagiola: None declared, M. Boscolo Anzoletti: None declared, A. El-Beshlawy: None declared, M. El-Alfy: None declared, V. Ramanan Madatha: None declared, P. Eshghi: None declared, R. Varadarajan: None declared, S. Hanagavadi: None declared, M. Karimi: None declared, M. V. Mangani: None declared, C. Ross: None declared, G. Young: None declared, T. Seth: None declared, S. Apte: None declared, D. Nayak: None declared, E. Santagostino: None declared, E. Mancuso: None declared, A. C. Sandoval Gonzalez: None declared, M. T. Owaidah: None declared, F. Rosendaal: None declared.

PO165-MON

Using HPSEC to quantify the effect of gamma-carboxylation on divalent metal-induced compaction of vkd-proteins and biological function

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Background: Factor IX (FIX) and Prothrombin are members of the vitamin K dependent (VKD) family of glycoproteins which have a conserved γ -carboxyglutamic acid (Gla) domain that contains four Ca^{2+} , three Mg^{2+} , and one interchangeable divalent-metal binding sites.

Aims: This study provides a quantitative solution phase perspective to the metal dependent conformational attributes of the VKD holoprotein. In contrast to previous studies using protein fragments, this study quantifies the compaction of Factor IX (FIX) and Prothrombin holoprotein by Ca^{2+} and Mg^{2+} .

Methods: High pressure size exclusion chromatography (HPSEC) was used to separate high and low Gla content recombinant (r-) FIX. Analytical ultracentrifugation (AUC) was used to calibrate HPSEC size change measurement and compared to predictions of size changes made by molecular modeling of X-ray crystallography data on VKD protein fragments.

Results: We observed a stark compaction of FIX and FII associated with Ca^{2+} and Mg^{2+} binding. The compaction induced by Ca^{2+} at physiological levels was equivalent to a change in the radius of hydration by AUC. The 5–6% compaction in the radius of gyration with sensitivity of ~ 0.01 nm between metal free and physiological levels of Ca^{2+} was equivalent to the predicted molecular modeling. In fully carboxylated FIX, compaction induced by physiologic levels of Ca^{2+} alone is greater than for Mg^{2+} alone. The presence of both divalent metals provided an additive or greater increase in compaction. Much less compaction was observed in low Gla r-FIX than in any other higher Gla FIX species. This compaction enables the separation of r-FIX species with high and low biological activity using HPSEC. FII exhibited similar divalent metal dependent compaction phenomena.

Conclusion: The divalent metal-induced holoprotein compaction phenomenon observed here primarily arises from conformational changes

in the core Gla domain associated with VKD protein biological activity.

Disclosure of Interest: N. Vanderslice: None declared, A. Messer: None declared, K. Vadivel: None declared, S. P. Bajaj: None declared, M. Phillips: None declared, M. Fatemi: None declared, W. Xu: None declared, W. Velander Shareholder of: ProGenetics, LLC.

PO166-MON

Increased immunogenicity of factor IX administered subcutaneously versus intravenously demonstrated in hemophilia B mice expressing a human major histocompatibility complex II haplotype

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Background: Although clinically challenging, factor IX inhibitors arise rarely in severe hemophilia B, which complicates the effort to study their pathogenesis and treatment.

Aims: To examine the association between inhibitor formation and the context of exposure to FIX.

Methods: Factor IX knockout (FIX^{-/-}) mice engineered to express the common human MHC II DR B1*1501 allele instead of mouse MHC II ($n = 10$ –11/group) were administered FIX weekly via one of three routes: subcutaneous (SC), intravenous (IV via tail vein), or via the retro-orbital venous plexus (RO) at a dose of 200 IU kg⁻¹ for 9 doses. FIX Bethesda inhibitor antibody was assessed at weeks 2, 4, 6, 8, and 12. At week 12, all the mice were sacrificed and evaluated for FIX binding immunoglobulins (IgG1 subclass).

Results: Totally 100% of mice that received weekly SC FIX replacement developed high titer FIX inhibitors by week 4–6 (>5 Bethesda inhibitor units, BU). These inhibitors persisted through week 12 with a mean titer of 25 BU mL⁻¹ (range 8–45 BU mL⁻¹). In contrast, weekly FIX replacement IV led to the development of low titer inhibitors (mean of positive values = 1.5 BU mL⁻¹, range 0.5–1.9 BU mL⁻¹); 38% developed inhibitors by week 4 and 75% by week 12. Twelve weekly RO exposures led to inhibitors in only 44% of the mice, all of which were near or below the lower level of sensitivity of the assay (mean of positives = 0.6 BU mL⁻¹, range 0.4–0.8 BU mL⁻¹).

Mice treated with SC FIX had a mean FIX-binding IgG1 level of 718 mcg mL⁻¹ (range 50–2170 mcg mL⁻¹), while the mice treated via the IV or RO route had a mean IgG1 level of 2 mcg mL⁻¹ and 0.05 mcg mL⁻¹, respectively.

Conclusion: Examined in mice humanized with respect to their MHC II haplotype, SC delivery of FIX clotting factor replacement is strongly immunogenic when compared to IV delivery, as shown by the generation of high titer inhibitors and FIX binding immunoglobulins. The observed timing of inhibitor development suggests that 3–4 weeks of the SC FIX exposure will be informative for examining pro-inflammatory signaling.

Disclosure of Interest: None declared.

PO167-MON

Pharmacokinetic (PK) and clinical data support for effectiveness of once-weekly recombinant coagulation factor IX (RFIX) dosing for prophylaxis in patients with moderately severe or severe hemophilia B

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Background: Two studies showed that rFIX (nonacog alfa) administered 100 IU kg⁻¹ once per week is effective as prophylaxis against

bleeding episodes in patients (pts) with moderately severe/severe hemophilia B (FIX activity [FIX:C] ≤ 2 IU dL⁻¹). Powell et al (NEJM 2013) reported that the half-life of FIX:C after rFIX administration was longer (33 h) than previously reported (18 h) when sampling duration continued to 96 h postdose.

Aims: Assess presence and impact of residual FIX:C late in the dosing interval during weekly prophylaxis dosing.

Methods: All available FIX:C data collected in 71 pts participating in both studies (NCT00364182, NCT01335061) were pooled and samples collected ~1 week (144–192 h) after a previous dose were assessed. Annualized bleed rate (ABR) was calculated per pt for each regimen. A post hoc survival curve analysis was performed using the Kaplan–Meier method comparing 100 IU kg⁻¹ once per week and 50 IU kg⁻¹ twice per week. Prophylaxis data were pooled by regimen. Failure was defined as 2 spontaneous joint bleeds during prophylaxis. If a second spontaneous bleeding episode occurred, date of this episode was used; otherwise, the pt was considered censored on date of the end of that regimen treatment period. Prophylaxis periods were 4 months (first study) and 12 months (second study).

Results: of 47 samples available, 30 showed FIX:C > 2 IU dL⁻¹ (median 4.0 IU dL⁻¹; min-max 2.0–11.4 IU dL⁻¹); consistent with a half-life of 33 h. Median ABR in 67 evaluable pts in the 100 IU kg⁻¹ once/week regimen was 1. Survival curve analysis of time from start of regimen until second spontaneous joint bleed showed no significant difference between regimens ($P = 0.42$).

Conclusion: Residual FIX:C ~ 1 week post dose support effectiveness of once-weekly prophylaxis rFIX in moderately severe/severe hemophilia B pts and that the half-life of rFIX is longer than previously reported.

Disclosure of Interest: J. Korth-Bradley Employee of: Pfizer, L. Smith Employee of: Pfizer, J. Fuiman Employee of: Pfizer, P. Rendo Employee of: Pfizer.

PO168-MON

Evaluation of host cell protein immunogenicity of a polished recombinant factor IX investigational product, IB1001 in previously treated patients with hemophilia B

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Background: IB1001 (trenonacog alfa) is a recombinant factor IX manufactured using Chinese Hamster Ovary (CHO) cells intended for management of hemophilia B. During clinical development, residual CHO protein (CHOP) antibody development (seroconversion) was detected in 31% (23/77) of clinical trial subjects. There were no adverse events associated with seroconversions or impact on efficacy in the subjects who seroconverted. An additional chromatographic step designed to further remove CHOPs was implemented and validated for manufacturing IB1001 (polished IB1001). Ongoing clinical trial subjects who provided informed consent were transitioned to polished IB1001.

Aims: To assess CHOP immunogenicity potential of polished IB1001 in clinical trial subjects with hemophilia B.

Methods: Ongoing subjects [adult, adolescent and pediatric previously treated patients with severe or moderately severe hemophilia B (factor IX activity ≤ 2 U dL⁻¹)] from two clinical trials ($n = 22$) were transitioned to polished IB1001 and tested for anti-CHOP at 5 exposure days (ED), 1 month, 3 months and every 3 months thereafter, after receiving polished IB1001. Twenty one subjects received polished IB1001 as prophylaxis (54 ± 16 IU kg⁻¹, twice weekly) and one subject on-demand (30 ± 3 IU kg⁻¹).

Results: All subjects who were negative for anti-CHOP ($n = 14$) or indeterminate (non-specific assay binding; $n = 1$) prior to transition to polished IB1001 remained negative/indeterminate after 3 months or 6 months ($n = 13$ negative, $n = 1$ indeterminate) of polished IB1001 treatment. Subjects, who were positive for anti-CHOP prior to transition to polished IB1001 ($n = 7$), displayed decreasing antibody titers at 3 months (7/7) or 6 months (4/4) of polished IB1001 treatment.

Conclusion: Polished IB1001 immunogenicity is reduced as demonstrated by absence of new seroconversions and decrease in anti-CHOP antibody titers in subjects who had previously seroconverted with former IB1001.

Disclosure of Interest: B. Drobic: None declared, Y. Hua: None declared, T. Babinchak: None declared, E. Gomperts Consultant for: Emergent BioSolutions, C. Hall: None declared.

PO169-MON

Factor IX immunogenicity in hemophilia B patients on replacement therapy with a recombinant factor IX investigational product (IB1001)

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Background: IB1001 (trenonacog alfa) is a recombinant factor IX manufactured using Chinese Hamster Ovary (CHO) cells intended for management of hemophilia B. During clinical development of IB1001, formation of binding antibodies (inhibitory and non-inhibitory) to factor IX (FIX) was monitored over time in previously treated patients with hemophilia B who received IB1001.

Aims: To monitor development of inhibitory and non-inhibitory anti-FIX antibodies in previously treated patients with hemophilia B who received IB1001.

Methods: Adult, adolescent and pediatric previously treated patients with severe or moderately severe hemophilia B (factor IX activity ≤ 2 U dL⁻¹) from two clinical trials ($n = 74$) were monitored for development of inhibitory anti-FIX antibodies (inhibitors) and non-inhibitory anti-FIX antibodies. Sixty five subjects received IB1001 as prophylaxis (54 ± 15 IU kg⁻¹, twice weekly) and 9 subjects received IB1001 on-demand (53 ± 18 IU kg⁻¹). Eight subjects had <50 exposure days (EDs), 59 had 50 EDs, while 49 reached 100 EDs. A subset of subjects ($n = 24$) received polished IB1001 (manufactured with a modified process to reduce host cell proteins). Twenty two subjects received polished IB1001 as prophylaxis (54 ± 16 IU kg⁻¹, twice weekly) and 2 subjects on-demand (34 ± 8 IU kg⁻¹); 13 subjects had <50 EDs and 11 subjects reached 50 EDs.

Results: Inhibitors were not detected in any of the 74 subjects at any time-point. Non-inhibitory anti-FIX antibodies were detected in 21/74 (28%) subjects. This antibody response was sporadic in all but three subjects and was not linked to any adverse events or lack of efficacy in any subject. Two (8%) subjects on polished IB1001 also displayed a non-inhibitory anti-factor IX sporadic response with no link to adverse events or lack of efficacy.

Conclusion: Hemophilia B subjects with long-term exposure to IB1001 did not develop inhibitors. Some subjects developed sporadic non-inhibitory factor IX antibodies with no clinical significance.

Disclosure of Interest: B. Drobic: None declared, Y. Hua: None declared, T. Babinchak: None declared, E. Gomperts Consultant for: Emergent BioSolutions, C. Hall: None declared.

PO170-MON

Indirect comparisons of factor consumption, bleeding rates, and infusion frequencies during routine prophylaxis with recombinant factor VIII Fc fusion protein and other recombinant factor VIII productsIorio A¹, Krishnan S², Myrén K-J³, Lethagen S^{3,4}, McCormick N⁵ and Karner P⁵¹McMaster University, Hamilton, Canada; ²Biogen Idec, Cambridge, USA; ³Sobi, Stockholm, Sweden; ⁴University of Copenhagen, Copenhagen, Denmark; ⁵Analysis Group, Boston, USA**Background:** There is a need to understand new long-acting recombinant factor VIII (rFVIII) treatments in the context of bleeding efficacy, infusion burden, and factor consumption (FC) during prophylaxis in patients with hemophilia A.**Aims:** We indirectly compared published clinical study results of annualized bleed rates (ABRs), FC, and infusion frequencies with rFVIII Fc fusion protein (rFVIII Fc) and conventional rFVIII products.**Methods:** A systematic literature review was conducted to identify clinical studies of routine prophylaxis with rFVIII in previously treated adults and adolescents with severe hemophilia A for comparison with rFVIII Fc (Mahlangu 2014, individualized arm, 1.4–2.4 infusions/week). Weekly FC, mean ABRs, and the number of weekly infusions were compared using simple differences between rFVIII Fc and other identified rFVIII products. Statistical comparisons could be conducted for studies reporting mean FC (alternatively, we conducted descriptive comparisons based on median FC reported or estimated from dose per infusion). Unreported standard deviations (SDs) of FC were estimated from the ratio of the SD to the mean in Mahlangu 2014.**Results:** Six studies met the inclusion criteria (2–4 infusions/week): 3 reported mean FC; comparisons for other studies were based on median FC. Mean weekly FC with rFVIII Fc (85.4 IU kg⁻¹) was lower vs. 2 rFVIII studies (Tiede 2014, $\Delta = 21.8$ IU kg⁻¹ [20%, $P < 0.001$]; Pollmann 2007, $\Delta = 15.5$ IU kg⁻¹ [15%, $P = 0.137$]) and higher vs. Lentz 2013, $\Delta = 14.3$ IU kg⁻¹ (20%, $P < 0.001$). Median weekly FC was lower with rFVIII Fc in all comparisons ($\Delta = 12.7$ – 29.8 IU kg⁻¹ [14–27%]). Mean ABR with rFVIII Fc was significantly lower vs. Tarantino 2004, Pollmann 2007, and Lentz 2013 (Δ ABR=3.1–3.6, $P < 0.001$) and comparable vs. other rFVIII studies.**Conclusion:** Indirect comparisons of clinical study results suggest that prophylaxis with rFVIII Fc may result in reduced FC with similar or improved bleeding rates and fewer infusions compared with other rFVIII products.**Disclosure of Interest:** A. Iorio Consultant for: Biogen Idec, Employee of: McMaster University, S. Krishnan Shareholder of: Biogen Idec, Employee of: Biogen Idec, K.-J. Myrén Employee of: Sobi, S. Lethagen Shareholder of: Sobi, Employee of: Sobi, University of Copenhagen, N. McCormick Consultant for: Biogen Idec, Employee of: Analysis Group, P. Karner Consultant for: Biogen Idec, Employee of: Analysis Group.

PO171-MON

Indirect comparisons of factor consumption, bleeding rates, and infusion frequencies during routine prophylaxis with recombinant factor IX Fc fusion protein and other recombinant factor IX productsIorio A¹, Krishnan S², Myrén K-J³, Lethagen S^{3,4}, McCormick N⁵ and Karner P⁵¹McMaster University, Hamilton, Canada; ²Biogen Idec, Cambridge, USA; ³Sobi, Stockholm, Sweden; ⁴University of Copenhagen, Copenhagen, Denmark; ⁵Analysis Group, Boston, USA**Background:** For patients with hemophilia B, prophylactic therapy with conventional factor IX (FIX) products requires 2–3 infusions/week, placing a burden on patients that may lead to poor adherence and suboptimal outcomes. There is a need to understand new long-acting recombinant FIX (rFIX) treatments in the context of bleeding efficacy, infusion burden, and factor consumption (FC) during prophylaxis.**Aims:** In the absence of head-to-head comparisons, FC, annualized bleed rates (ABRs), and infusion frequencies during routine prophylaxis with recombinant FIX Fc fusion protein (rFIX Fc) and conventional rFIX products were indirectly compared based on reported clinical study results.**Methods:** A systematic literature review was conducted to identify clinical studies of routine prophylaxis with rFIX in previously treated adults and adolescents with severe hemophilia B for comparison with rFIX Fc (Powell 2013, weekly prophylaxis). Weekly FC, mean ABR, and the number of weekly infusions were compared using simple differences between rFIX Fc and conventional rFIX products from identified studies. FC comparisons were based on the mean, reported or estimated from the dose per infusion. Unreported standard deviations (SD) of FC were estimated from the ratio of the SD to mean in Powell 2013.**Results:** Three studies met the inclusion criteria (1 to >3 infusions/week). Mean weekly FC with rFIX Fc (46.3 IU kg⁻¹) was significantly lower vs. rFIX based on Roth 2001, Lambert 2007, and Windyga 2014 (difference=42.8–74.5 IU kg⁻¹, [48–62%], $P < 0.001$). The mean ABR for rFIX Fc of 3.1 was numerically lower vs. rFIX based on comparisons with Roth 2001, Lambert 2007, and Windyga 2014, but the differences (0.0–2.4 bleeds/year) were not statistically significant.**Conclusion:** Based on indirect comparisons of clinical study results, once weekly infusions with rFIX Fc may be associated with significantly lower FC and similar bleeding rates compared with more frequently-infused rFIX products.**Disclosure of Interest:** A. Iorio Consultant for: Biogen Idec, Employee of: McMaster University, S. Krishnan Shareholder of: Biogen Idec, Employee of: Biogen Idec, K.-J. Myrén Employee of: Sobi, S. Lethagen Shareholder of: Sobi, Employee of: Sobi, University of Copenhagen, N. McCormick Consultant for: Biogen Idec, Employee of: Analysis Group, P. Karner Consultant for: Biogen Idec, Employee of: Analysis Group.

PO172-MON

Safety and efficacy of a novel AAV vector for treatment of hemophilia BAnguela X^{1,2}, Toso R², Couto LB², Chen Y², Hui D², DiPietro M², Lee B¹, Qu G², Hauck B², Corbau R^{1,2}, Wright F² and High KA²¹The Children's Hospital of Philadelphia; ²Spark Therapeutics, Inc, Philadelphia, USA**Background:** Liver-targeted delivery of AAV2/8 vectors encoding human factor IX (hFIX) has achieved recent clinical success for the treatment of hemophilia B (HB). However, significant challenges

remain. While the clinical improvement in patients who achieved stable hFIX levels of around 5% of normal is indisputable, risk for excessive hemorrhage after trauma or surgery would be significantly reduced if stable levels were closer to 50%. Also, at least 50% of patients are not eligible for AAV8 treatment due to the presence of neutralizing antibodies (NABs) that will block hepatocyte transduction.

Aims: The goal of this study was to evaluate the efficacy and safety of a novel bioengineered capsid (AAV-Spark100) containing the high specific activity hFIX-Padua variant.

Methods: FIX antigen and activity were monitored in non-human primates. NAB prevalence was measured in serum from HB patients.

Results: NAB prevalence against several AAV capsids was screened in ~60 patient sera. The % of samples with a NAB titer <1:1 was 58.9% for AAV-Spark100 vs. 45.3% for AAV8, indicating that ~10% more patients could potentially be treated with this novel capsid. Studies in non-human primates comparing the potency of AAV8 and AAV-Spark100 showed comparable hepatic expression of hFIX, as well as similar biodistribution profiles. Using AAV-Spark100 expressing the hFIX-Padua transgene at a dose of 1×10^{12} g kg⁻¹, stable hFIX activity levels of up to ~35% of normal were achieved 3 months after treatment. There was no evidence of thrombosis in any animals, even those injected with a five fold higher dose and expressing as much as 350% normal hFIX activity, suggesting that this novel vector does not pose significant thrombogenicity risks. No vector-related changes in the hematology or clinical chemistry parameters were observed. Histopathological analysis revealed no gross or microscopic findings and no evidence of thrombosis.

Conclusion: Results from this study support the safety and efficacy of AAV-Spark100-hFIX-Padua hepatic gene transfer.

Disclosure of Interest: None declared.

PO173-MON

Comparison of fix activity of select novel recombinant fix replacement products in commonly used fda approved one-stage clot assay systems

Robinson MM, Tiefenbacher S, Ross EL, Williams PT, Cogswell CE, Wham TL and Adcock DM

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Background: Reagent dependent recoveries for novel rFIX replacement products have been described for FIX one-stage (OS) clotting assays.

Aims: A side by side comparison of select novel rFIX replacement products was performed utilizing the most common FDA-approved, OS FIX assay systems employed in the U.S. The percent recoveries of three novel rFIX products were compared to BeneFIX[®] (Wyeth Pharmaceuticals Inc.).

Methods: FIX OS activity was determined for each product (based on labeled potency) at 80, 30, 15, 5 and 1% activity spiked into hemophilia B plasma (HRF, Inc.). Samples were tested in triplicate following manufacturer recommended protocols, calibration and QC, on three separate occasions, in four FDA approved assay systems: STA-R Evolution[®] using PTT A and C.K. Prest[®] (Diagnostica Stago Inc.), BCS[®] XP using Dade[®] Actin[®] FSL (Siemens Healthcare) and ACL TOP[®] using HemosIL[®] SynthASil[®] (Instrumentation Laboratory). Recoveries were also assessed by chromogenic (CS) assay on the STA-R Evolution[®] using BIOPHEN Factor IX.

Results: With the exception of the BCS[®] XP, all FIX OS assay systems showed some degree of non-linearity (i.e. increasing recovery with decreasing product concentration). Only two of the FIX products (ALPROLIX[®] and BeneFIX[®]), recovered within $\pm 25\%$ in at least one of the FIX OS assay systems, across all concentrations tested. Recoveries for individual products across the four FIX OS assay systems

were highly variable. In the FIX CS assay, recoveries between rFIX products were less variable, with all novel rFIX products recovering within $\pm 20\%$ at the 80% concentration and N9-GP recovering within $\pm 20\%$ across all concentrations tested.

Conclusion: Factor recovery for individual FIX products (including BeneFIX[®]) was highly assay system dependent, with only one of the three novel rFIX replacement products recovering within $\pm 25\%$ in one of the FIX OS systems. Clinical laboratories need to be aware of the reagent dependent recovery of rFIX products.

Disclosure of Interest: M. Robinson: None declared, S. Tiefenbacher Consultant for: Novo Nordisk, E. Ross: None declared, P. Williams: None declared, C. Cogswell: None declared, T. Wham: None declared, D. Adcock Consultant for: Novo Nordisk.

PO174-MON

Moyamoya syndrome in a hemophilia A patient with a high-titre FVIII inhibitor

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Background: The development of alloantibodies against factor VIII is considered the most significant complication in patients with hemophilia A as it is associated with severe bleeding complications. The majority of inhibitors develop at a young age that is not prone to thromboembolic events in general. We present the unique case of a 2 years old boy with severe hemophilia A who developed an inhibitor against factor VIII and experienced 3 strokes between 17 and 24 months of age.

Aims: We aimed to elucidate the mechanism of recurrent strokes in our hemophilia A patient.

Methods: We carefully analysed thrombophilic risk factors and performed extensive neurovascular imaging. In addition we analysed the FVIII gene in detail using Sanger sequencing and MLPA.

Results: Analysis of the patients data for stroke risk factors remained inconclusive. Inhibitor tolerance induction treatment (ITI) used high doses of FVIII/VWF concentrates according to the Bonn protocol. One stroke occurred during treatment with bypassing agents. Despite careful dosing of bypassing agents and inhibition of platelet function using salicylic acid 2 additional strokes occurred after one and 6 months, respectively.

Neurovascular imaging showed high-grade stenosis of both carotid arteries at the T-junction and reduced vessel calibres of additional medium size cerebral arteries resembling moyamoya-syndrome. Genetic analysis revealed a complex intron 22-inversion with deletion of the BRCC3 gene. Loss of BRCC3 is a known cause of Moyamoya syndrome.

Conclusion: Complex rearrangements of intron 22 in the FVIII gene can lead to a novel syndrome with association of hemophilia A and Moyamoya syndrome. The treatment of patients with this syndrome can be extremely challenging. In our case conventional ITI failed. Neither careful dosing of bypassing agents nor inhibition of platelet function prevented additional cerebral insults. Despite a high risk for bleeding complications, a surgical intervention might be necessary to prevent further strokes.

Disclosure of Interest: None declared.

PO175-MON

Optimizing procoagulant activity for mutant recombinant factor IX with resistance to heparin- and antithrombin

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Background: Antithrombin (AT) is the primary plasma inhibitor of FIXa and markedly accelerated by heparin. Mutations in the heparin- (K126A or K132A) and AT-binding (R150A) exosites of FIXa selectively disrupt these interactions. FIX(a) K126A/R150A and K132A/R150A demonstrate preserved plasma thrombin generation, reduced AT-heparin inhibition, and prolonged in vitro plasma half-life.

Aims: The aim of these studies was to assess the impact of the R170A mutation on pro-coagulant functions of FIX K126A/R150A and K132A/R150A.

Methods: Human FIX cDNA constructs with alanine substitutions (chymotrypsinogen #) in the heparin- (K126A, K132A, R170A) and AT-binding (R150A) exosites were stably transfected into HEK293 cells and proteins purified from conditioned media. Proteins were characterized in APTT-based coagulant and tissue factor or FIXa-initiated plasma thrombin generation assays (TGA).

Results: For APTT-based coagulant activity in FIX deficient plasma, % clotting activities for FIXa (normalized to WT) were: FIX R170A (659 ± 32), R150A/R170A (520 ± 14), K132A/R170A (359 ± 24), K126A/R170A (331 ± 15), R126A/R150A/R170A (166 ± 8), WT (104 ± 9), pFIXa (106 ± 7), R150A (62 ± 4), and R132A/R150A/R170A (17 ± 2). Coagulant activity for the zymogens demonstrated a similar trend. For the FIXa-initiated TGA, % peak thrombin values (normalized to WT) were: R150A/R170A (322 ± 24), R170A (298 ± 27), K126A/R170A (189 ± 17), K132A/R170A (140 ± 20), R126A/R150A/R170A (134 ± 9), WT (100 ± 13), pFIXa (88.9 ± 20), and K132A/R150A/R170A (1.6 ± 0.9). Peak thrombin values for the zymogens followed a similar trend.

Conclusion: The R170A mutation enhanced plasma coagulant activity and TGA for FIX K126A/R150A. In contrast, addition of R170A to FIX K132A/R150A significantly reduced plasma coagulant activity and dramatically disrupted plasma TG. Optimization of pro-coagulant activity for FIX proteins with reduced AT- and heparin inhibition should enhance therapeutic efficacy in hemophilia B.

Disclosure of Interest: None declared.

Disseminated intravascular coagulation

PO176-MON

To evaluate the utility of ISTH scoring system, aptt and schistocytes as markers of severity in cases of disseminated intravascular coagulation

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Background: Disseminated Intravascular Coagulation (DIC) is an acquired disorder with intravascular activation of coagulation, excessive thrombin formation, fibrin deposition in microcirculation and consequent ischemic organ damage. The ISTH scoring system provides a quantitative indicator of severity and prognosis while aPTT, detection of Schistocytes form important part of laboratory investigations.

Aims: To evaluate the utility of ISTH DIC scoring system, aPTT and Schistocytes as markers of severity in cases of DIC.

Methods: Eighty-seven cases of clinically suspected DIC were studied over a period of 18 months. Complete blood counts with study of

peripheral blood smear for presence of Schistocytes, Platelet count, PT, aPTT, D-dimer, Plasma Fibrinogen were analysed. Impact of ISTH DIC scores, derangement of aPTT values and presence of Schistocytes on patient outcome was studied. Chi square and Fisher's exact tests were used for analysis of the data.

Results: There was a significant association of ISTH score with outcome of the patients ($P < 0.05$). 58 (66.7%) patients had ISTH DIC score of < 5 suggestive of non-overt DIC and 29 (33.3%) patients had a score of ≥ 5 consistent with overt DIC. A high score ≥ 5 was associated with a poor outcome in 13 patients (45%). Low DIC score was associated with increased chances of survival ($P < 0.001$). Raised aPTT and presence of Schistocytes showed a significant association with a high ISTH score. 19 (21.8%) patients showed presence of schistocytes; out of which 17 patients had DIC score ≥ 5 hence statistically significant ($P < 0.001$). The aPTT values were deranged in 58 patients. Out of these, 26 patients were of overt DIC making it statistically significant ($P < 0.008$).

Conclusion: ISTH DIC scoring criteria help to quantify severity of DIC. Derangement of aPTT value and presence of Schistocytes correlate with the ISTH DIC score and are also useful predictors of severity.

Disclosure of Interest: None declared.

PO177-MON

Role of plasma high mobility group box 1 in disseminated intravascular coagulation with leukemia

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Background: High mobility group box 1 (HMGB1) is a DNA-binding protein acting as a proinflammatory cytokine when released by necrotic cells, monocytes or macrophages. It also plays a role in the coagulation activation and several tumors including leukemia. DIC with leukemia is treated as a special branch and has less targeted molecular markers for its early diagnosis.

Aims: The objective of this study was to investigate the role of HMGB1 in the diagnosis of DIC with leukemia.

Methods: Eighty nine blood samples from leukemia patients in Wuhan Union Hospital were prospectively included in the study. Among them, 83 cases were suspected of DIC, while the other 6 were the negative controls. Their clinical data, laboratory tests and plasma samples were collected or measured respectively. Accordingly, we made scores for these patients by Chinese DIC Scoring System (CDSS).

Results: In this study, we found that in leukemia patients, levels of plasma HMGB1 were higher in the DIC group than non-DIC ($121.13 \text{ ng mL}^{-1}$ vs. 60.30 ng mL^{-1} , $P = 0.001$). And along with the increase of DIC score, the levels of HMGB1 increased gradually ($P < 0.01$). The sensitivity of HMGB1 for DIC diagnosis was high ($\text{Se} = 93.33\%$), and there was a tendency of increased HMGB1 levels in pre-DIC. Finally, the HMGB1 levels declined slowly during the recovery stage, and HMGB1 levels were not significantly different between 28-day survivors and non survivors.

Conclusion: The increased levels of plasma HMGB1 were related tightly to the diagnosis and severity of DIC in leukemia patients. Furthermore, the increased HMGB1 levels may help for the early diagnosis of DIC. Therefore, HMGB1 is a potential targeted molecular marker for the diagnosis of DIC with leukemia.

Disclosure of Interest: None declared.

PO178-MON

Role of activated protein c and soluble fibrin in the early phase of trauma with disseminated intravascular coagulation: comparison with acute coagulopathy of trauma-shock

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Background: Disseminated intravascular coagulation (DIC) is characterized by insufficient anticoagulation mechanism-induced thrombin generation not localized to the injured sites.

Aims: We hypothesized that activated protein C does not increase in DIC after trauma, which leads to systemic thrombin generation followed by a poor outcome and that the same is true in acute coagulopathy of trauma-shock (ACOTS).

Methods: We conducted a prospective cohort study of 57 trauma patients subdivided into 30 with DIC and 27 without DIC based on the Japanese Association for Acute Medicine criteria. Patients with ACOTS, defined as a prothrombin time ratio > 1.2, were also evaluated. Twelve healthy volunteers served as controls. The levels of activated protein C and soluble fibrin were measured on days 1, 3, and 5 after admission. The outcome measures were organ dysfunction by a Sequential Organ Failure Assessment score and the 28-day mortality.

Results: On day 1, normal to decreased activated protein C and increased soluble fibrin were observed in patients with DIC to a greater degree than in the controls and non-DIC patients. These changes were more significant in those who simultaneously met the International Society on Thrombosis and Haemostasis overt DIC criteria and were associated with poorer outcome. Regression analyses with multiple and logistic methods showed the DIC scores and soluble fibrin levels on day 1 to be independent predictors of organ dysfunction and death in the DIC patients. The changes in the measured variables in ACOTS patients coincided with those in DIC patients.

Conclusion: Normal to decreased activated protein C is considered to play role in systemic thrombin generation at an early phase of trauma, which is associated with a worse outcome of DIC patients. The same is true in patients with ACOTS, and no shutoff of thrombin generation was observed.

Disclosure of Interest: None declared.

PO179-MON

Evaluation of new Chinese diagnostic scoring system for disseminated intravascular coagulation

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Background: DIC is complicated and no single clinical or laboratory test has an adequate sensitivity and specificity to confirm or reject a diagnosis of DIC. It is still a challenging work needing abundant professional experience to diagnose DIC accurately.

Aims: To evaluate the sensitivity and specificity of the new Chinese DIC scoring system (CDSS).

Methods: This study retrospectively collected the information of 619 patients (371 cases with non-hematologic malignancies, 248 cases with hematologic malignancies) who suspected of DIC in Wuhan Union Hospital during 2013-4 to 2014-6. We validated CDSS by comparing with three leading scoring systems, from ISTH, JAAM and JMW, and evaluated its prognostic value by 28 days mortality, APACHE II and SOFA score.

Results: In non-hematologic malignancies, CDSS was more specific than JAAM (72.55% vs. 50.49%, $P < 0.05$) and more sensitive than ISTH (77.07% vs. 62.03%, $P < 0.05$). In hematologic malignancies, the area under the ROC curve of CDSS was larger than ISTH and JMW (0.933 vs. 0.889, $P < 0.01$ with ISTH, 0.944 vs. 0.845, $P < 0.01$ with JMW). In addition, the 28-day mortality rate, SOFA scores, APACHE II scores of DIC patients diagnosed by CDSS were significantly greater than non-DIC ($P < 0.05$).

Conclusion: We are the first group to propose CDSS. It emphasized the values of the clinical manifestations, the rapidly declining platelet count, APTT in the diagnosis of DIC and used D-dimer as the fibrin-related maker. DIC with hematological malignancies was treated as a special part. In this study we can see that CDSS displayed an acceptable property for the diagnosis of DIC with appropriate sensitivity and specificity, and also had a good prognostic value for DIC patients.

Disclosure of Interest: None declared.

PO180-MON

A novel murine model of disseminated intravascular coagulation (DIC) to aid the development of novel therapeutics to treat DIC

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Background: Disseminated intravascular coagulation (DIC) is a complex clinical condition that results in the activation of both the coagulation and fibrinolytic systems, resulting in simultaneous thrombosis and bleeding. Current DIC treatment regimes are unsuccessful and as a consequence up to 10-50% of cases result in death as a consequence of multiple organ failure.

Aims: To develop a murine disseminated intravascular coagulation (DIC) model to aid the development of novel therapeutics to treat DIC.

Methods: Thrombus formation was induced by the stimulation of the cremaster muscle of C57BL/6 mice with TNF- α for 4 h followed by LPS challenge (i.v). Mice were anaesthetized by a ketamine/xylazine mixture and the trachea and the carotid artery were cannulated. The cremaster muscle was exteriorized and prepared for microscopic observations. LPS (100 μ g) was injected via the carotid artery and the effects on post-capillary venules were studied every 10 min for 1 h.

Results: Clot formed in the post-capillary venules of TNF- α primed mice within 10 min of LPS administration and peak at 30 min (approximately 4% of total vessel occlusion). Mice receiving TNF- α or LPS alone had significantly reduced levels of aggregation. LPS resistant mice (C3H/HeJ) had reduced aggregation and no haemorrhagic injury compared to control mice. Electron microscopy imaging suggested clots to consist mainly of leukocytes and platelets.

Conclusion: We conclude that understanding the mechanisms of DIC and having a murine model of DIC will aid the development of novel therapeutics that have the potential to treat DIC in the future.

Disclosure of Interest: None declared.

PO181-MON

The presence of overt disseminated intravascular coagulation at diagnosis of adult patients with acute myeloid leukemia

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Background: Disseminated intravascular coagulation (DIC) often develops in adult acute promyelocytic leukemia (APL) patients. In adult non-APL acute myeloid leukemia (AML) patients, DIC develops rarely. There are few data about the presence of overt DIC at diagnosis in other types of AML or among different cytogenetic and molecular genetics AML prognostic subgroups.

Aims: We wanted to find out any significant difference in type of DIC between different prognostic and age groups.

Methods: The coagulation tests, cytogenetics and molecular genetics data were available for 105 (48 females, 57 males) out of 222 (109 females, 112 males) adult non-APL AML patients diagnosed between January 2008 and December 2011. The median age of patients was 59, range from 20 to 81 years. The overt DIC was assessed at diagnosis by calculating ISTH DIC score. According the European LeukemiaNet (ELN) standardized reporting system for genetic abnormalities, patients were categorized into four risk groups: favorable, intermediate-I, intermediate-II, and adverse (ELN risk subgroups). Chi-square test was carried out to compare the qualitative variables. A *P* value less than 0.05 was considered to be significant.

Results: The ISTH overt DIC score was found in 33.3% (35/105) of non-APL adult AML patients. There was no statistically significant difference in type of DIC between ELN risk subgroups (*P* = 0.895). There was no statistically significant difference in type of DIC between patients below or under 60 years old (*P* = 0.300).

Conclusion: The percentage of adult non-APL AML patients with the overt DIC at diagnosis was similar or even higher comparing with the available data from the literature (approximately 15%). It seems that age or ELN risk subgroups are not related with the incidence of the overt DIC.

Disclosure of Interest: None declared.

PO182-MON

Biomarker profile of sepsis-associated coagulopathy using biochip assays for inflammatory cytokinesWalborn A¹, Hoppensteadt D¹, Syed D¹, Abro S¹, Mosier M² and Fareed J¹*¹Pathology; ²Surgery, Loyola University Medical Center, Maywood, USA*

Background: Disseminated intravascular coagulation (DIC) is one of the major pathophysiological aspects of sepsis and greatly increases the risk of death in sepsis patients. DIC involves activation of both the coagulation and fibrinolytic processes along with the generation of inflammatory cytokines.

Aims: The purpose of this study was to analyze the levels of inflammatory cytokines in the plasma of patients with DIC in order to compare with normal individuals.

Methods: Plasma samples from 36 patients with sepsis and/or suspected DIC and 48 normal individuals were included. A biochip array to profile IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN γ , TNF α , IL-1 α , IL-1 β , MCP-1, and EGF was used (Randox, Antrim, UK). The results were compared using an unpaired *t* test.

Results: The biochip array analysis showed an elevation of IL-6, IL-8, IL-10, VEGF, IFN γ , TNF α , IL-1 β , MCP-1, and EGF in DIC patients which was statistically significant compared to normal controls (*P* < 0.005). IL-6, and IL-8 showed particularly large increases in

patient's plasma relative to levels in normal levels. Correlations were made between the percent change from the normal mean for each cytokine for each DIC sample. Spearman correlation coefficients were used with α = 0.05 as a cutoff for significance.

Conclusion: The inflammatory biomarkers IL-1 β , IL-6, IL-8, IL-10, IFN γ , VEGF, TNF α , MCP-1, and EGF showed significant elevation in patients with DIC compared to normals. Additionally, significant correlations were found between these particular markers and each other as well as with other measured inflammatory markers. It is interesting to note that while numerous significant correlations were present between IL-1 β , IL-6, IL-8, IL-10, IFN γ , TNF α , MCP-1 and many of the inflammatory cytokines measured, VEGF and EGF exhibited much less extensive correlation, suggesting that their involvement in DIC may be separately caused and regulated than that of the other investigated inflammatory markers.

Disclosure of Interest: None declared.

Endothelium / vessel wall I

PO183-MON

Hypoxia induces dysregulation of MIR-126 and affects VEGF AND MMP-9 expression in endothelial cellsFujii S¹, Ogasawara M² and Iwaki S²*¹Laboratory Medicine, Asahikawa Medical University, Asahikawa; ²Pathobiology, Nagoya City University, Nagoya, Japan*

Background: miR-126 is the miRNA specially expressed in endothelial cells and is associated with angiogenesis.

Aims: The role of miR-126 in hypoxia-inducible angiogenesis and the potential underlying mechanism were investigated.

Methods: The expression of miR-126 was measured in EA.hy926 endothelial cell line under hypoxia (1% O₂, 0-48 h) using real-time PCR. The miR-126 was increased or decreased by transfecting miR-126-mimics or inhibitors to cells. The mRNA levels of vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) were assessed by real-time PCR.

Results: The expression of miR-126 was decreased by 40% with hypoxia at 24 h compared with normoxic condition (*n* = 4, * *P* < 0.05 vs. 0 h). Brief exposure to hypoxia for 6 and 12 h exerted the same effects and decrease in miR-126 was not recovered at 24 h (*n* = 4, * *P* < 0.05 vs. control). The expression of VEGF mRNA was increased by 5-fold in hypoxia-induced cells (**P* < 0.05 vs. 0 h, *n* = 4). The expression of MMP-9 mRNA was increased by 2.5-fold in hypoxia-induced cells (**P* < 0.05 vs. 0 h, *n* = 3). The VEGF mRNA levels were unaffected in hypoxia-induced cells pretreated with miR-126-mimics and increased in the cells pretreated with miR-126-inhibitors. The MMP-9 mRNA levels were increased in hypoxia-induced cells pretreated with miR-126-mimics and unaffected in the cells pretreated with miR-126-inhibitors.

Conclusion: miR-126 is persistently down-regulated for up to 24 h with only a brief hypoxic condition in vitro. The effects of miR-126 on the expression of VEGF may differ from the effects on the expression of MMP-9, exhibiting the widely varying roles of miR-126 in hypoxia-inducible neovascularization.

Disclosure of Interest: None declared.

PO184-MON

Antioxidant pharmacological strategies for the prevention of endothelial dysfunction in uremia

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Background: Accelerated atherosclerosis in Chronic kidney disease (CKD) is preceded by the development of endothelial dysfunction (ED), with the instauration of a proinflammatory and prothrombotic phenotype and enhanced oxidative stress.

Aims: We evaluated the effect of nutraceuticals with anti-inflammatory action and synthesized antioxidant compounds in an *in vitro* model of uremia induced ED.

Methods: Endothelial cells (ECs) were pretreated with the flavonoids apigenin, genistein and quercetin, and the antioxidant enzyme mimetics ebselen, EUK-134 and EUK-118. After 16 h, ECs were exposed to medium containing sera from patients on peritoneal dialysis (PD, $n = 10$) or from healthy donors (C, $n = 15$). Changes in the expression of the adhesion receptor ICAM-1 and the production of intracellular reactive oxygen species (ROS) were assessed by fluorescence. Activation of the inflammation-related proteins p38 MAPK and NFkB were evaluated by ELISA and Western-blot.

Results: Exposure of ECs to uremic media resulted in a significantly increased expression of ICAM-1, overproduction of ROS and increased activation of NFkB and p38MAPK ($P < 0.05$ PD vs. control experiments). Pretreatment with ebselen, EUK 134, and EUK118 inhibited ICAM-1 expression and ROS generation generated by uremic conditions ($P < 0.01$ vs. PD). In relation to flavonoids, only quercetin showed a moderated but significant inhibitory effect on both parameters ($P < 0.05$ vs. PD). All the compounds assayed reduced p38MAPK uremia-induced activation ($P < 0.05$ vs. PD). Only the antioxidant-enzyme mimetics were able to inhibit the activation of NFkB produced by the uremic media. ($P < 0.05$ vs. PD).

Conclusion: These results indicate that the antioxidant enzyme mimetics exhibit not only antioxidant but also anti-inflammatory effects on the endothelium. Therefore, reduction of oxidative stress may be an efficient strategy in the prevention of ED in uremia.

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Disclosure of Interest: None declared.

PO185-MON

Anti-thrombotic and pro-fibrinolytic effects of levosimendan in human endothelial cells in vitro

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Background: Levosimendan is an inodilator for the treatment of acute decompensated heart failure (HF). Data from clinical studies suggest that levosimendan is particularly effective in HF due to myocardial infarction. After acute revascularization, no reflow-phenomenon is a common complication that may lead to pump failure and cardiogenic shock.

Aims: Our aim was to examine whether levosimendan interferes with the pro-thrombotic phenotype of activated endothelial cells *in vitro*.

Methods: Human heart microvascular endothelial cells (HHMEC) and human umbilical vein endothelial cells (HUVEC) were treated with interleukin-1b (IL-1b) (200 U mL⁻¹) or thrombin (5 U mL⁻¹) and co-

treated with or without levosimendan (0.1–10 µM) for 2–24 h. Effects on plasminogen activator inhibitor-1 (PAI-1) and tissue factor (TF) expression and activity were measured by rt-PCR, specific ELISA and flow cytometry.

Results: Treatment with IL-1b or thrombin significantly increased the expression of PAI-1 and TF in endothelial cells. Co-treatment with levosimendan strongly attenuated the effects of IL-1b and thrombin on PAI-1 and TF mRNA by up to 50% and 45%, in a dose- and time-dependent manner. Furthermore, co-treatment with levosimendan dampened the antigen production of PAI-1 and the surface expression of TF by 35% and 45%, respectively. Additionally, levosimendan diminished both TF and PAI-1 activity.

Conclusion: Levosimendan down-regulates the expression of the pro-thrombotic and anti-fibrinolytic biomolecules TF and PAI-1 in activated human endothelial cells. Our findings may, at least in part, explain some of the beneficial effects of levosimendan after myocardial reperfusion.

Disclosure of Interest: None declared.

PO186-MON

RNA-SEQ analysis of human major liver cell types reveals differential expression profile of sinusoidal versus other hepatic vascular endothelial cells

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Background: Besides playing role in liver physiology, sinusoidal endothelial cells (SEC) are central to many pathological processes. These cells are characterized with their special morphology and function driving from their unique expression profile.

Aims: Unraveling expression profile and analyzing major biological pathways in Liver SECs comparing with those of other vascular ECs of the Liver as well as hepatocytes can provide a better understanding of liver physiology in health.

Methods: We have used Illumina RNA-seq to profile the transcriptome of hepatocytes, CD31 + ECs isolated from the whole liver parenchyma and FACS-sorted SECs based on the co-expression of Tie2 and CD32b. Libraries were prepared according to the standard Illumina TruSeq RNA sample preparation protocol and sequenced using an Illumina HiSeq 2000 generating 50 bp reads. TopHat/Cufflinks standard pipeline was used to align the short reads to the coding regions and to measure gene expression.

Results: The expression profile of SECs is clearly distinguishable from ECs of the Liver vasculature with about 1500 genes that are differentially expressed, more than 10 fold, in Tie2 + /CD32b+ versus CD31 + ECs isolated from the whole liver parenchyma. Comparing with hepatocytes, more than 800 genes were only expressed in Tie2 + /CD32b+ cells while more than 2000 genes were identified to be only expressed in hepatocytes.

Conclusion: Unlike other vascular endothelial cells, CD31 which is widely used for isolation of all types of ECs, is expressed on LSECs at a very low level. Therefore, using this marker for LSEC isolation results in a pool of ECs from the Liver parenchyma including the specialized liver SECs. In this study, we confirmed liver SEC isolation by labeling CD32b in the population of Tie2 + ECs provides a pure population of sinusoidal ECs. Besides, distinct biological pathways specific

to these cells and/or to ECs isolated from the rest of the liver vasculature as well as hepatocytes are identified.

Disclosure of Interest: None declared.

PO187-MON

Endothelial fibrinolytic response onto an evolving matrix of fibrin

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Background: Fibrin provides a temporary matrix on the site of vascular injury.

Aims: Follow fibrin formation and lysis at the surface of human dermal microvascular endothelial cells (HMEC-1).

Methods: Fibrin formation (at different fibrinogen concentrations) or lysis (triggered by a supplement of tPA and plasminogen) on the surface of endothelial cells was followed by measuring changes in absorbance (350 nm). Clot structure was visualized by laser scanning confocal microscopy (LSCM). The secretion of uPA and PAI 1 were quantified from the supernatant of HMEC-1 monolayer in contact with fibrin by ELISA.

Results: In the presence of cells, both the rate of fibrin formation and final turbidity were slightly higher compared to the control without cells. The HMEC-1 retarded significantly clot dissolution at all fibrinogen concentration tested (0.5–3 mg mL⁻¹). UPA secretion was ~1.3 ng mL⁻¹ in the presence of fibrin, without RGD, compared to 0.8 ng mL⁻¹ with RGD, when clots were formed on the top of HMEC-1 (model 1). However, the opposite was found when cells were grown on the top of fibrin (model 2): 0.6 ng mL⁻¹ without RGD vs. 1.7 ng mL⁻¹ with RGD. PAI 1 secretion was independent to the presence of fibrin/RGD (~700 ng mL⁻¹, model 1; 500 ng mL⁻¹, model 2).

Conclusion: The HMEC-1 had a subtle effect on fibrin formation but delayed clot degradation. Thrombin had no effect on PAI 1 secretion at 1 nM.

Disclosure of Interest: None declared.

PO188-MON

A microfluidic vascular injury model using heat-activated endothelial cells

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Background: Endothelial cells (EC) inhibit and promote platelet activation in a shear stress dependent manner. Activated EC can promote platelet adhesion by secretion of VWF, exposure of thrombogenic proteins and expression of selectins. Previous studies of EC-platelet interactions use chemical activators to homogeneously activate EC. In this study, we focally activate EC using heat, creating a well-defined injury zone of activated EC that are surrounded by EC inhibiting platelet activation.

Aims: 1) To develop an in vitro heat-induced focal injury that can differentially activate and kill EC. 2) To characterize EC activation and platelet accumulation following heat injury.

Methods: Human umbilical vein EC (HUVEC) were grown to confluence in microfluidic channels (100 × 1000 µm) for 48–72 h. Endothelialized microfluidic channels were placed on top of thermally

evaporated Cr/Au (10 nm/100 nm) electrodes and 5–9 V were applied for 30 s. Whole blood was perfused over the EC monolayer for 7 min at 150 s⁻¹ and platelet aggregate volumes were measured. P-selectin expression, viability, and VWF release from the EC were quantified. An infrared thermal camera and finite element model estimated the rise in surface temperature.

Results: Microelectrodes induced peak temperature increases of 10–40 °C above 37 °C after applying 5–9 V. At a 14 °C increase, there was no EC activation or platelet accumulation. An 18 °C increase lead to P-selectin positive EC directly on top of the electrodes. Platelets accumulated in the retracted spaces between P-selectin positive EC. Temperatures exceeding an increase of 22 °C resulted in EC death and no platelet accumulation. Experiments with blocking antibodies suggest that platelets adhere between activated EC via GPIbα-VWF and α₆β₁-laminin interactions.

Conclusion: We have developed a focal vascular injury model using resistive heating by microelectrodes to differentially produce zones of dead and/or activated EC. Platelet adhesion appears to be supported by EC-derived VWF and laminin.

Disclosure of Interest: None declared.

PO189-MON

Evidence of defibrotide internalization and its endothelial protective effect in hepatic endothelial in vitro model

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Background: At October 2013, Defibrotide (DF) received EMA authorization for its use in the treatment of severe hepatic veno-occlusive disease, a clinic syndrome suspicious to have an endothelial dysfunction origin. As DF interferes with several steps of the coagulation-inflammation cycle and has a recognized role as endothelial protective agent it is also susceptible to be tested in other complications with an endothelial origin. For that the uncovering of its mechanism of action is needed.

Aims: To investigate the interaction of DF with a hepatic endothelial cell line from human origin (SK-HEP-1) and the mechanisms involved in its traffic.

Methods: SK-HEP-1 cells were exposed to DF (4 µg mL⁻¹), previously labelled with ULYSIS® Nucleic Acid Labeling Kits, for up to 24 h. Using inhibitory assays and flow cytometry techniques along with confocal microscopy, we have explored: 1) DF internalization, and dose-response kinetics, and 2) different pathways of endocytosis.

Results: Flow cytometry assays revealed concentration, temperature and time dependent up-take of DF by Sk-HEP-1 cells. Moreover, inhibitory assays indicate that entrance of DF into endothelial cells occurs primarily through macropinocytosis, and that this mechanism seems to be highly dependent on actin assembly followed by endosome traffic. Confocal microscopy allowed visualization of significant interaction of DF with endothelial cell membranes followed by internalization and redistribution to the cytoplasm. DF did not reach the cell nucleus even after 24 h of exposure.

Conclusion: Our studies show that DF interacts with endothelial cells membranes, becoming internalized and redistributed into endothelial cell compartments without evidence in the nucleus. Our findings may contribute to a better understanding of the precise mechanisms of action of DF as a therapeutic and potential preventive agent on the endothelial damage underlying different pathological situations.

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PO190-MON

Circulating endothelial cells are increased in post-thrombotic syndrome patients

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Background: Post-thrombotic syndrome (PTS) is a complication of lower limbs deep vein thrombosis (DVT). Circulating endothelial cells (CEC) are derived from vascular wall or bone marrow, and have been related to hemostasis.

Aims: CEC, D-dimer and IL8 were analyzed in PTS patients ($N = 19$, all of them presenting residual thrombi, grouped according to Villalta scale), in DVT patients with no PTS ($N = 9$) and to healthy individuals (CTR, $N = 19$, matched to PTS patients by gender, ethnic origin and age ± 5 years).

Methods: Flow cytometry analyzes were performed including CD31, CD144, CD133, anti-VEGFR2 and CD45. Plasma and serum were used on inflammatory markers analyses.

Results: Mild PTS patients presented higher levels of CEC using CD144 (0.06) and anti-VEGFR2 (0.121) in comparison to DVT patients (CD 144: 0.019, $P = 0.015$; VEGFR2: 0.032, $P = 0.019$) and to CTR (CD 144: 0.026, $P = 0.077$; VEGFR2: 0.034, $P = 0.04$). Moderate+severe PTS patients presented higher levels of CEC using anti-VEGFR2 (0.109) in comparison to DVT patients (0.032, $P = 0.008$) and to CTR (0.034, $P = 0.013$). Using CD144, the increased amounts were almost significant between PTS patients (0.044) and DVT patients (0.019, $P = 0.054$). D-dimer was increased in patients with residual thrombi but without PTS (0.85, $P = 0.009$) and also in mild SPT patients (0.5, $P = 0.03$) in comparison to CTR (0.31). Mild PTS patients (26.46) and moderate+severe PTS patients (13.84, $P = 0.03$) presented different levels of IL8 in comparison to DVT without PTS patients (20.28, $P = 0.03$ and $P = 0.02$, respectively). CEC did not correlate to IL8 or D-dimer levels.

Conclusion: Different levels of D-dimer on residual thrombi patients with no PTS and in mild PTS patients are probably due to lower levels of thrombi fibrosis. Different levels of IL8 and CEC in PTS patients may be related to angiogenic properties and increasing tendency on oxidative stress situations. Probably CEC play a role on PTS pathophysiology, certainly associated to the endothelial injury.

Disclosure of Interest: None declared.

PO191-MON

Endothelial dysfunction after hematopoietic cell transplantation (HCT) is enhanced in acute graft-versus-host disease (GVHD)

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Background: Acute GVHD is a leading cause of non relapse mortality after allogeneic HCT. We demonstrated endothelial dysfunction in association with allogeneic HCT that could be responsible for several associated early complications.

Aims: The potential role of the endothelial damage in the development of GVHD was evaluated.

Methods: Human endothelial cells were grown in the presence of serum from patients receiving allogeneic HCT, non developing (-GVHD, $n = 12$) or developing GVHD on days +7 to +14 (+GVHD14, $n = 6$) or days +14 to +21 (+GVHD21, $n = 6$). Blood samples were collected before conditioning (Pre), before transplantation (day 0), and at days 7, 14, 21 and 28 after transplantation. We evaluated changes in the expression of VCAM-1 on cell surfaces, and VWF on the extracellular matrix (ECM), by immunofluorescence, and the reactivity of the ECM towards platelets, after being exposed to citrated blood ($800s^{-1}$, 5 min). Plasma VWF was analyzed, by ELISA, as a biomarker of endothelial dysfunction.

Results: Expression of VCAM-1, presence of VWF and platelet adhesion onto the ECM were markedly elevated when cells were exposed to +GVHD14 and +GVHD21 conditions in comparison to -GVHD. These markers reached maximum values in response to sera collected at GVHD diagnose (increases with respect to -GVHD of 8.8%, 35.0% and 20.6%; and of 10.7%, 40.0% and 20.8% for VCAM-1, VWF and platelet adhesion in +GVHD14 and +GVHD21, respectively). Plasma VWF was always higher in GVHD patients, specially at diagnose (increases of 37.7% and 100.3% in +GVHD14 and +GVHD21 vs. -GVHD).

Conclusion: Our studies indicate that humoral factors from allogeneic HCT recipients with GVHD induce a more marked proinflammatory and prothrombotic action on endothelial cells in culture than those present in recipients who do not develop GVHD. Our results could contribute to establish a pattern of early biomarkers with predictive value on the development of GVHD.

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PO192-MON

Effect of maternal anti-HPA-1A antibodies on endothelial cellsDardik R^{1,2}, Rosenberg N^{1,2}, Loewenthal R³ and Salomon O^{2,4}¹National Hemophilia Center, Sheba Medical Center, Tel Hashomer; ²Sackler School of Medicine, Tel Aviv University, Tel Aviv; ³Tissue Typing Laboratory; ⁴Thrombosis and Hemostasis Unit, Sheba Medical Center, Tel Hashomer, Israel

Background: Neonatal alloimmune thrombocytopenia (NAIT) is caused by maternal antibodies directed against fetal platelet antigens of paternal origin. The majority of NAIT cases result from incompatibility in HPA-1 located on the $\beta 3$ integrin subunit, which forms a complex with the αIIb integrin subunit on the platelet surface (integrin $\alpha IIb\beta 3$ [GPIIb/IIIa]) and with the αv integrin subunit (integrin $\alpha v\beta 3$) on the endothelial cell (EC) surface. The most severe complication of NAIT is fetal/neonatal intracranial hemorrhage (ICH). Since no correlation has been observed between the risk of ICH and the severity of neonatal thrombocytopenia, endothelial damage caused by binding of maternal anti-HPA-1a antibodies to ECs has been suggested to play a role in ICH.

Aims: To investigate the effect of anti-HPA-1a antibodies present in the sera of mothers genotyped as HPA-1bb and with a history of NAIT, on ECs.

Methods: Serum samples were collected from 10 mothers genotyped as HPA-1bb with a history of NAIT, shortly after delivery. Confluent layers of ECs (HUVEC) were incubated with 10% heat-inactivated maternal serum for 24 h. RNA extracted from ECs exposed to maternal sera positive for anti-HPA-1a antibodies was examined for expression of genes involved in apoptosis by quantitative real-time PCR using a commercial gene array.

Results: Serum of 1 (KZ) out of the 10 mothers' sera examined caused partial detachment of ECs and increased expression of the pro-apoptotic genes Bax, FADD and GADD45 alpha, whereas sera of the remaining 9 mothers showed no effect on EC adhesion or expression of genes involved in apoptosis. Mother KZ delivered a baby with a platelet count of 5000 mL⁻¹, which was genotyped as HPA-1ab. Of note, she received IVIG during her next pregnancy, but did not respond to IVIG treatment: platelet count of the second baby born following IVIG treatment was also 5000 mL⁻¹.

Conclusion: Endothelial damage may be caused by maternal anti-HPA-1a antibodies in isolated cases of NAIT.

Disclosure of Interest: None declared.

PO193-MON

Endothelial dysfunctions in cirrhosis patientsRadu CM, Bulato C, Rodriguez-Castro K², Zerbinati P, Saggiorato G, Senzolo M² and Simioni P¹Department of Medicine²Multivisceral Transplant Unit, Department of Surgery, Oncology and Gastroenterology, University of Padua, Padua, Italy

Background: The risk of thromboembolic events in patients with cirrhosis is reported to be two-fold higher than in controls, due to the imbalance between pro and anti-coagulant factors. Endothelial dysfunction is a key initiating event in the etiology of vascular diseases and has been linked to the pathogenesis of thrombosis in cirrhotic patients. Thrombosis has been reported to be a consequence of imbalance of functional phenotypes, such as thrombosis markers of the endothelial cell surface, which is involved in the procoagulant and anticoagulant pathways.

Aims: We analyzed the expression of the endothelial coagulation proteins in samples of vena cava and portal vein from cirrhosis patients, in order to evaluate endothelial dysfunction.

Methods: Vena cava and portal vein samples were obtained from 15 cirrhosis patients at transplant surgery and during organ retrieval in 15 non-cirrhosis patients (controls). The fragment of the veins samples were immediately conserved in buffered formalin. We analyzed by immunofluorescence techniques the expression of the major important coagulation endothelial markers.

Results: We observed a low or absent expression of the most important anti-coagulant endothelial proteins: thrombomodulin, endothelial protein C receptor and tissue factor protein inhibitor. In the contrary, we observed an increased expression of tissue factor and E-selectin which indicated endothelial activation.

Conclusion: These results directly demonstrate the presence of endothelial dysfunction in the liver vasculature of patients with cirrhosis, suggesting an activation of the endothelium. This study on endothelial cells could give us the possibility to observe in situ the endothelial damage present in cirrhosis and clarify the possible mechanism underlying the splanchnic thrombosis occurrence.

Disclosure of Interest: None declared.

PO194-MON

Kindlins are actin binding proteinsBialkowska K, Bledzka K, Pluskota E, Qin J and Plow EF
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Background: The kindlins are a family of cytoskeletal proteins that have received considerable attention for their capacity to bind to and regulate the function of integrins. In mammals, the kindlin family consists of three members (kindlin-1, -2, -3), each encoded by a separate gene. Human diseases that arise from the deficiencies of kindlin-1 or kindlin-3 have been attributed to disruption of extracellular matrix-integrin-actin networks. Kindlin-2 is lethal early in embryonic development when its gene expression is ablated in mice. The observations made in mice and zebra fish with reduced kindlin-2 levels also emphasize its localization in focal adhesion-like structures and its role in linking integrins to the actin cytoskeleton.

Aims: To investigate the direct interaction of kindlins with actin and to assign the functional significance to this interaction.

Methods: The in vitro binding assays using purified proteins and cell-based assays were used.

Results: Reduction in kindlin-2 levels in endothelial cells, in mouse C2C12 using siRNA, or in kindlin-3 levels in human erythroleukemia cells using siRNA disorganizes the actin cytoskeleton and decreases the spreading on $\beta 3$ integrin substrates. These changes led us to consider direct binding between kindlins and actin. Indeed, direct interaction of purified kindlin-2 and kindlin-3 with F-actin was observed in co-sedimentation experiments. In surface plasmon resonance, actin bound kindlin-2 in a concentration and time dependent manner. Addition of kindlin-2 to F-actin induced formation of actin bundles, also indicative of direct interaction. We have begun to locate the actin binding site(s) in kindlins. Pull-down assays of kindlin-2 derivatives indicated that an integrin binding site in F3 was not essential for actin binding. SPR showed that an actin binding site resided in the amino-terminal region of kindlin-2.

Conclusion: Kindlins are actin binding proteins, and this finding provides a new way to interpret their role of kindlins in signaling across integrins.

Disclosure of Interest: None declared.

Fibrinogen / fibrin / D-dimer I

PO195-MON

Evaluation of different assays of D-DIMER and soluble fibrin in emergency department patients with suspected venous thromboembolism

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Background: D-dimer (DDI) is a heterogeneous degradation product of cross-linked fibrin and named the biochemical gold standard for excluding suspected Venous Thromboembolism (VTE). Specificity is low since levels increase with e.g. age, pregnancy, cancer, trauma, inflammation and infection, so imaging techniques are needed to verify diagnosis. Soluble Fibrin (SF), activated but not yet polymerized fibrin, is more specific to intravascular coagulation than is DDI and may be preferred in some settings.

Aims: Comparing assays of DDI and SF for identifying VTE in symptomatic out-patients.

Methods: Five hundred and seventy eight patients with clinically suspected VTE were recruited from the medical emergency department of Karolinska University Hospital (inclusion still ongoing). VTE was verified by imaging techniques (ultrasonography, computed tomography or ventilation/perfusion lung scintigraphy, as appropriate). Non-VTE was identified by imaging techniques or prolonged clinical follow-up. VTE was verified in 15% ($n = 91$); 29% of these developed pulmonary embolism. DDI and SF concentrations were measured in citrated plasma, using two instruments (Sysmex CS2100i and Stago CompactMax).

Results:

PO196-MON

Performance of age adjusted D-dimer cut off value: a step towards improving clinical utility in diagnosis of asymptomatic venous thromboembolism

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Background: Asymptomatic venous thromboembolism (VTE) is a leading problem in elderly hospitalized patients, it presents a difficulty in diagnosis and consumes both patient and hospital resources. Old age is accompanied with an increase in D-dimer level that is not associated with VTE, leading to increased investigations in elderly due to its poor specificity.

Aims: To explore the reliability of conventional D-dimer cutoff value ($500 \mu\text{g L}^{-1}$) against the age-adjusted cutoff formula (patient's age $\times 10 \mu\text{g L}^{-1}$) when excluding VTE in patients over 65 years.

Methods: From an initial pool of 1123 patients, 252 patients aged >65 years were selected. Those 252 patients had shown decreased mobility after hospital admission. Laboratory and radiological results were collected from the patients' charts in Ain Shams University hospitals. Patients were divided into 3 groups according to their age; first group 65–70 years, second group 71–80 years and third group above 81 years. Conventional cutoff sensitivity and specificity were compared to age-adjusted cutoff sensitivity and specificity in the different groups.

Results: Patients had mean age of 73.3 (± 6.74). Of those patients, 35.7% showed compression changes in their Doppler examination and were diagnosed with VTE. Patients in the first group, conventional cutoff showed sensitivity 100% and specificity 37.3% whereas age-adjusted formula showed 100% and 49.5% respectively. Second group, conventional cutoff showed sensitivity 100% and specificity 24.2% whereas age-adjusted formula showed 100% and 51.4% respectively. In third group, conventional cutoff showed sensitivity 100%

	Efficiency (%)	Sens (%)	Spec (%)	NPV (%)	PPV (%)	LR(-)	LR(+)	Kappa
Roche Tina-quant DDI (CS2100i) [$<0.25 \text{ mg L}^{-1}$]	70	94	65	98	33	0.1	2.7	0.409
Siemens INNOVANCE DDI (CS2100i) [$<0.25 \text{ mg L}^{-1}$]	48	100	38	100	23	0	1.6	0.160
MediRox DDI (CS2100i) [0.20 mg L^{-1}]	68	87	65	97	31	0.2	2.5	0.297
STA-Liatest DDI (CompactMax) [$<0.50 \mu\text{g mL}^{-1}$ FEU]($n=155$)	60	96	52	99	31	0.1	2.0	0.252
STA-Liatest FM SF (CompactMax) [$<5.2 \mu\text{g mL}^{-1}$ FEU]($n=109$)	54	63	52	87	22	0.7	1.3	0.088
STA-Liatest FM SF(CS2100i) [$<9 \text{ mg L}^{-1}$]	83	38	91	89	44	0.7	4.2	0.304

NPV/PPV= Negative/Positive predictive value, LR (-/+) = Negative/Positive Likelihood Ratio.

Conclusion: The heterogeneity of the measurand and chosen cutoffs may explain the deviating performance of the assays. A combination of a DDI with a maximized sensitivity, and a SF, or other biomarker, with high specificity (see table) for VTE may provide a combined diagnostic performance as an alternative to imaging.

Disclosure of Interest: None declared.

and specificity 52.6%, whereas age-adjusted formula showed 97% and 35.2% respectively.

Conclusion: Using the age-adjusted cutoff formula when interpreting D-dimer results will lead to better specificity when diagnosing VTE in patients ages 65–80 years, while in patients over 80 years, the more sensitive conventional cut off is preferred.

Disclosure of Interest: None declared.

PO197-MON

Evaluation of the ratios of high molecular weight fibrin degradation products (HMW FDP) to d-dimer in patients' blood by the immunoassay equally recognizing D-dimer and HMW FDPKogan A¹, Mukharyamova K¹, Bereznikova A¹, Koshkina E², Kara A¹ and Katrukha A¹¹Hyttest, Turku, Finland; ²City Hospital N 67, Moscow, Russia

Background: Fibrin clot lysis in blood results in the formation of a broad range of fibrin degradation products (FDP) of different molecular weights including the smallest one - D-dimer (DD). The information about the ratios of high molecular weight FDP (HMW FDP) to DD in patients' blood is limited since the commercial assays have different specificity to various forms of FDP. We suggest that the assay with equal specificity to all FDP forms could help to evaluate the real ratios of HMW FDP to DD in patients' blood.

Aims: Our goal was to develop mAbs equally recognizing HMW FDP and DD and utilize them in immunoassay to evaluate the ratios of HMW FDP to DD in blood of patients with different diseases.

Methods: mAbs specific to DD were obtained by traditional hybridoma technique. Two mAbs equally recognizing HMW FDP and DD were selected and used in the fluoroimmunoassay to quantify DD and HMW FDP levels in plasmas of patients with deep vein thrombosis ($n = 5$), sepsis ($n = 7$), and one day after the surgery ($n = 6$). Plasma samples were separated by gel filtration using AKTA-FPLC system, and concentrations of FDP forms in fractions were determined by the newly-developed assay.

Results: mAbs DD189 and DD255 with equal specificity to HMW FDP and DD were obtained. Assay utilizing these mAbs (DD189 as capture and DD255 as detection MABs) also recognized equally both forms of FDP. It was shown that in blood of thrombotic patients FDP levels prevailed over DD levels (up to 3.5 times). In septic patients FDP and DD levels were comparable, whereas in patients after the surgery DD levels often exceeded FDP levels (up to 2.5 times). In blood of patients with the same disease the FDP/DD ratios were not constant and could vary several times.

Conclusion: The newly-developed assay DD189-DD255 with equal specificity to HMW FDP and DD enable evaluating precisely the FDP/DD ratios in patients' plasmas. The clinical value of this ratio should be analyzed.

Disclosure of Interest: None declared.

PO198-MON

Isochronous design to study the stability of liquid D-dimer controls

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Background: Human fibrin digested products partially purified and stabilized have been used to prepare liquid D-Dimer controls for the HemosIL® D-Dimer HS 500 (Instrumentation Laboratory, Bedford, MA, USA) assay at two levels, one around the clinical cut-off for Venous Thromboembolism (500 ng mL⁻¹ FEU) and another well above the cut-off. The classical design to study controls stability is a long-term testing of material placed at the recommended storage temperature and tested at different time points throughout the study duration. This design is sensitive to long-term variability. An alternative proposal is the isochronous design where samples withdrawn from the storage conditions are placed monthly below -70 °C until the end of the study.

Aims: To study the stability of liquid HemosIL D-Dimer HS 500 Controls at the recommended storage conditions (2–8 °C) with an isochronous design.

Methods: The stability has been studied following an isochronous staggered end design. A sufficient number of vials from three lots of controls were placed at 2–8 °C at the start of the study until the check day, freezing some of them below -70 °C each month until the end of the study (19 months). Then, all vials and a set of vials kept at -70 °C from day 0 are thawed and measured randomly. With this design, data is generated simultaneously at the end of the study. Stability was assessed by a linear regression model of the percent recovery versus time.

Results: As all the regression slopes were statistically significant, stability was defined as the time at which the lower limit of the 95% confidence interval (CI) of the regression line intercepts the control recovery at the 85% acceptance criterion. These values were higher than 19 months for all the samples. The lower sided 95% CI intercepted the 19 months of testing with recoveries ranging between 88.9% and 93.7%.

Conclusion: An isochronous design to test stability of liquid HemosIL D-Dimer HS 500 Controls demonstrated a shelf life of 19 months.

Disclosure of Interest: None declared.

PO199-MON

The factors associated with elevated D-dimer level in patients with acute venous thromboembolismVorobyeva N¹, Dobrovolsky A¹, Titaeva E¹, Ermolina O¹, Balakhonova T¹, Kirienko A² and Panchenko E¹¹Russian Cardiology Research and Production Complex; ²Russian State Medical University, Moscow, Russia

Background: D-dimer (D-D) is a well-investigated marker for diagnosis of venous thromboembolism (VTE).

Aims: To determine factors associated with elevated D-D level in pts with acute VTE.

Methods: One hundred and eleven pts (76 men) in the age 18–76 (mean 54 ± 14) years with deep vein thrombosis (DVT) and/or pulmonary embolism (PE) were included in the study. Diagnosis of DVT was confirmed by compression ultrasonography. "Size" of thrombus was calculated in points using our own scale. Diagnosis of PE was confirmed by ventilation-perfusion scintigraphy, multi-detector computed tomographic angiography or pulmonary angiography. D-D was measured by a quantitative assay with reagent kits «STA LIATEST D-DI» (Diagnostica Stago) with estimated cut-off level of 0.5 µg mL⁻¹. At admission to hospital, the duration of DVT/PE symptoms was ranged from 1 to 60 days.

Results: Median of D-D level was 1.05 (interquartile range 0.49–1.99) µg mL⁻¹. D-D level exceeded the upper limit of normal level in 74% of pts. Median of the duration of DVT/PE symptoms was 32 (interquartile range 23–44) days. D-D level had a positive correlation with "size" of thrombus ($r = 0.32$; $P = 0.001$) and a negative correlation with the duration of DVT/PE symptoms ($r = -0.42$; $P < 0.001$). We also studied other factors, which may influence on D-D level in pts with acute VTE. Multivariate analysis showed that female (OR 8.38; 95% CI 1.91–36.80; $P = 0.005$), chronic heart failure (OR 17.31; 95% CI 1.69–177.25; $P = 0.016$), the duration of DVT/PE symptoms less 30 days (OR 5.44; 95% CI 1.54–19.26; $P = 0.009$) and "size" of thrombus over 6 points (OR 13.61; 95% CI 3.43–54.01; $P < 0.001$) are independent predictors of elevated D-D level in pts with acute VTE.

Conclusion: In pts with acute VTE (mean duration of DVT/PE symptoms was 32 days), D-D level was elevated in 74% of them. Except "size" of thrombus and the duration of DVT/PE symptoms, two clinical factors (female and chronic heart failure) were associated with elevated D-D level.

Disclosure of Interest: None declared.

PO200-MON

Baseline D-DIMER levels as a predictor for recanalisation in patients with first episode of deep venous thrombosis (DVT) of the lower limb

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Background: Recanalization is the process through which an occluded thrombus undergoes changes in size, shape and structure resulting in eventual re-establishment of the venous lumen. Various factors are known to influence recanalisation rates in patients with lower limb DVT.

Aims: Analyze the recanalisation rates in patients with DVT of the lower limbs and also evaluate the various factors, especially whether baseline D-Dimer levels effect this recanalisation

Methods: Twenty five patients (16males/9females) ≥ 18 years of age (37.67 ± 13.3 years) with first episode of DVT of the lower limb were enrolled. All but one patient received subcutaneous at the dose of 1 mg kg⁻¹ body weight at the time of diagnosis. Only one patient received unfractionated heparin infusion. In all 25 patients warfarin was started simultaneously at a dose of 5 mg taken and the initial treatment with heparin continued for at least 5 days or until an international normalized ratio (INR) of >2 was maintained for at least 48 h. For diagnosis (n=25) of DVT and to assess recanalisation, a duplex ultrasound of the lower limbs was done after one (n=20) and 3 months (n=17) of anticoagulation. A quantitative baseline D Dimer levels were measured in 14 patients using the STA latest D-dimer kit with STA compact analyzer. The mean D-dimer levels in these patients at baseline patients was 2.96 ± 4.9 mg mL⁻¹ (range 0.2–10 mg mL⁻¹).

Results: At 1 and 3 months re-evaluation we observed of 40limbs/20 patients(1 month) and 34limbs/17 patients(3 months), 40%/16.6% remained in the state of complete occlusion, 5%/16.6% completely recanalised, 55%/66.8% partial recanalised. We observed better recanalisation rates both at one(66.7% vs. 50%) and 3 months(100% vs. 66.7%) in patients with baseline D Dimer levels of <0.5 IU L⁻¹ as compared to the patients with a baseline D Dimer levels of ≥ 0.5 IU L⁻¹.

Conclusion: Although our study size was small but baseline D-Dimer levels have a definite role in predicting recanalisation in patients with DVT of the lower limbs.

Disclosure of interest: None declared.

PO201-MON

An evaluation of the TOSOH bioscience ST AIA-PACK Ddimer performed with the AIA-360 automate

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Background: DDimer is a degradation product formed as a result of the action of plasmin on cross-linked fibrin. Raised DDimer levels are observed in diseases and conditions associated with increased coagulation activation and can also be used in the exclusion of thromboembolic events (VTE) eg. deep vein thrombosis or pulmonary embolism.

Aims: An evaluation of the TOSOH ST AIA-PACK D-Dimer test.

Methods: The TOSOH ST AIA-PACK DDimer is a 2 site immuno-enzymometric assay performed for the purpose of this study on the TOSOH AIA 360 instrument.

Our study included:

- 1 An assessment of the overall suitability of the test system including inter- / intra-assay reproducibility and linearity
- 2 A comparison of data with that obtained using Siemens Innovance, for samples from subject groups including; Normals ($n = 20$), exclusion of VTE ($n = 35$) and investigation of possible DIC ($n = 42$)

Results: The inter assay CVs at three levels (mean DDimer values of 350, 570 & 2810 ng mL⁻¹) ranged from 2.2 to 3.5%, and intra assay CVs at two levels (mean DDimer values of 480 & 7329) ranged from 3.6 to 4.1%. The linearity based on two plasma samples with measured DDimer concentrations of approximately 7600 and 14 000 gave 'R' values of 0.9985 and 0.9988

Significant differences were observed between the means of the TOSOH and Siemens Systems Ddimer result in the Normal and VTE exclusion subject groups, $P < 0.0001$. Correlation of the two methods gave 'r' values of 0.7798, 0.9693, 0.9574 (excluding two outliers) and 0.9713 when comparing results in Normal, VTE exclusion, possible DIC subject groups and 'All' clinical groups combined, respectively.

Conclusion: The TOSOH system is a user friendly, robust system with acceptable levels of reproducibility and linearity over a wide concentration range (up to 16 000 ng mL⁻¹ without the need for pre analysis dilution). In agreement with manufacturer's recommendations it would be necessary for each laboratory to determine a reference interval corresponding to the characteristics of the population being tested.

Disclosure of Interest: None declared.

PO202-MON

evaluation of diagnostic accuracy of two D-dimer assays in pulmonary embolism

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Background: The choice of the best D-dimer assay may be a challenge for the laboratory because the assays on the market vary in their sensitivity and specificity. In the diagnosis of pulmonary embolism (PE), the ability of the D-dimer assay to minimize unnecessary imaging procedures may be a criteria of choice.

Aims: To compare the diagnostic accuracy of two D-dimer assays in outpatients suspected of PE.

Methods: Seventy banked samples from patients enrolled in a previous study (# NCT00368836) were tested with STA[®] - Liatest[®] D-Di, Stago (DD1) and VIDAS[®] D-Dimer Exclusion[™], Biomérieux (DD2).

Seventeen patients with confirmed PE (based on imaging results) and 53 PE negative patients (based on imaging results or 90-day follow-up) were tested.

Patients were included if they presented at least one symptom indicative of proximal deep venous thromboembolism or PE. Patients were not included if they had anticoagulant therapy, pregnancy, cancer or surgery.

Correlations of D-dimer results obtained with the two reagents were analyzed using linear regressions and Bland Altman plot. Diagnostic agreement to clinical evidence was shown through 2×2 tables.

Results: Linear regression was: $y = 1.1331x - 0.2091$ ($r = 0.908$).

Three samples had discrepant D-dimer results but were all "D-dimer false positive" (D-dimer level $\geq$ clinical cut-off but final diagnosis of the patient was "no PE").

Sensitivity was 94.1% for both assays. All true PE positive cases were identified, except one (76 years old patient diagnosed positive for subsegmental PE confirmed by CTPA).

Specificity was 45.3% for DD1 and 24.5% for DD2.

Conclusion: D-dimer levels are well correlated with the two assays.

In addition, sensitivity of these assays in the diagnosis of PE is equivalent. Finally, DD1 assay was able to identify more true negative cases than DD2 and could therefore suggest that less patients would undergo unnecessary imaging procedures.

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Fibrinolytic system

PO203-MON

DS-1040: a novel selective inhibitor of activated form of thrombin-activatable fibrinolysis inhibitor

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Background: DS-1040 is a low-molecular weight inhibitor of the activated form of thrombin-activatable fibrinolysis inhibitor (TAFIa) and is now under development for the treatment of thrombotic diseases.

Aims: To establish the potency and selectivity of DS-1040 as a TAFIa inhibitor by in vitro profiling.

Methods: (1) Inhibitory effects of DS-1040 on human, rat, and monkey TAFIa were evaluated using plasma from each species. (2) Effects of DS-1040 on platelet aggregation induced by ADP, TRAP, collagen, and U-46619 were assessed using human platelet-rich plasma. (3) Effects of DS-1040 on PT and APTT were assessed using normal pooled human plasma. (4) Effects of DS-1040 on various receptors, channels, transporters (a total of 68 items), and enzymes (a total of 53 items) were examined using radioligand binding assays.

Results: (1) DS-1040 inhibited human, rat, and monkey TAFIa activity with IC₅₀ values of 5.92 nmol L⁻¹, 8.01 nmol L⁻¹, and 4.48 nmol L⁻¹, respectively. (2) The IC₅₀ values of DS-1040 for platelet aggregations induced by ADP, TRAP, collagen, and U-46619 were estimated to be greater than 100 μmol L⁻¹. (3) The concentrations of DS-1040 to increase PT ratio and aPTT ratio to 1.15 were estimated to be greater than 100 μmol L⁻¹. (4) DS-1040 inhibited the binding of radioligand to phosphodiesterase 4 (PDE4) with IC₅₀ value of 4.57 μmol L⁻¹. However, no inhibition of enzyme activity was noted on the major PDE4 subtypes at concentrations up to 100 μmol L⁻¹. DS-1040 had no effects on any of the other receptors, channels, transporters, and enzymes at a concentration of 10 μmol L⁻¹.

Conclusion: DS-1040 is a potent and selective TAFIa inhibitor.

Disclosure of Interest: K. Noguchi Employee of: Daiichi Sankyo Co., Ltd., N. Edo Employee of: Daiichi Sankyo Co., Ltd., Y. Ito Employee of: Daiichi Sankyo Co., Ltd., T. Maejima Employee of: Daiichi Sankyo Co., Ltd., K. Yamaguchi Employee of: Daiichi Sankyo Co., Ltd.

PO204-MON

The state of fibrinolytic system in athletes at Russian north

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Background: The increase in blood fibrinolytic activity during stress is associated with increased secretion of oxytocin, which stimulates fibrinolysis by increasing the activity of plasmin, enhancing the action plasminogen activators and oppression of antiplasmin. During the increasing both the lasting and intensity of exposure to the stressor (regardless of its nature) balance in the reactions of coagulation and fibrinolytic systems are gradually replaced by the growing hypercoagulability.

Aims: In the process of analyzing of stressful changes in the hemostatic system, we can note one side of these changes - the hypercoagulation offensive that presents a risk of thrombosis.

Methods: The study involved 98 sportsmen who practice various sports activities. Estimated parameters of fibrinolysis: tissue plasminogen activator, Urokinase plasminogen activator and complex plasmin - antiplasmin.

Results: Based on the results obtained with the 95% probability we can say that the level of tissue plasminogen activator in athletes, is in the range of 0.61 - 0.88 ng mL⁻¹. Urokinase plasminogen activator level ranged from 0.96 to 9.66 (Me = 1.78) ng mL⁻¹. U-PA level in 50% athletes was within the limits 1.3–3.13 ng mL⁻¹. Adopted reference range of 1.2–2.4 ng mL⁻¹. The level of plasmin-antiplasmin complex varies from 10.21 to 260.79 ng mL⁻¹ (Me = 88.45). In 50% of athletes plasmin-antiplasmin complex determined within 41.87–187.03 ng mL⁻¹. By virtue of what possible to judge on the absence of pathological changes in the level of plasmin-antiplasmin complex. A breakdown of athletes on kind of sports and sports skills (depending on the sports category) was carried out.

Conclusion: Thus, our results indicate the presence of inhibition of the fibrinolytic system in athletes, manifested in the reduction of tissue plasminogen activator. However, the normal and in some cases, elevated levels of urokinase-type plasminogen activator may suggest compensation of low level of t-PA.

Disclosure of Interest: None declared.

PO205-MON

MIR-30C regulates thrombus formation in type 2 diabetic mellitus

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Background: Patients with type 2 diabetes (DM2) have a highly increased risk of thrombotic complications with cerebrovascular disease and severe peripheral vascular disease. High levels of PAI-1 have been reported in DM2, and miR-30c could directly regulate platelet-derived PAI-1 in DM2, however, the mechanisms by which miR-30c mediates thrombus formation in DM2 are less well understood.

Aims: Our aim was to determine the role of miR-30c in the regulation of arterial thrombosis.

Methods: The physiological relevance of miR-30c in arterial was studied in the high-fat diet (HFD)-fed mice that induced PAI-1 over-expression. To assess the effect of miR-30c on thrombosis, we used a carotid artery FeCl₃ injury model after the lenti-miR-30c injection.

Results: After 3 min by FeCl₃ injury, the blood flow was recorded by the Color Laser Doppler Image scanner. The carotid arterial occlusion time in HFD-fed mice was shorter than that of WT mice (122 ± 20 vs. 211 ± 25 s, *P* < 0.05). Strikingly, after the lenti-miR-30c injection, the carotid occlusion time in HFD-fed mice (260 ± 30 s) or WT mice (395 ± 35 s) were significantly increased compared with lenti-NC mice respectively (lenti-NC-HFD-fed, 150 ± 21 s and lenti-NC-WT, 245 ± 26 s).

Conclusion: These results identify an important role for miR30c in controlling thrombus formation in DM2, indicating targeting of miRNA representing a novel therapeutic opportunity for treating thromboembolism disease under DM2.

Disclosure of Interest: None declared.

PO206-MON

The influence of the peptide PRO-ARG-GLY on the regulation of the fibrinolytic and insular systems of the body on the model of diabetes mellitus type 2

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Background: The regulatory peptides glyproline family when entering the body have antiplatelet, anticoagulant, fibrinolytic and hypoglycemic effects in the blood. L-arginine has a positive effect on insulin secretion, increases the sensitivity of peripheral receptors to him, reducing the risk of developing diabetes type 2, which means negative changes in the hemostatic system.

Aims: To examine the fibrinolytic and hypoglycemic activity of the peptide Pro-Arg-Gly applied intranasally with the development of type 2 diabetes mellitus (T2DM).

Methods: The experiment was carried out on white rats. They modeled diet-induced T2DM: daily for 2 weeks they intragastrically injected 40% glucose solution at a dose of 0.5 mL per 200 g of body weight, and with 12 days intranasally injected with a solution of peptide PRG dose of 1 mg kg⁻¹ 2 times a day. Control rats with T2DM instead peptide was administered an equal volume of saline. Blood on the survey was taken 1 h after the last injection PRG and after 6 days of cancellation. In the blood plasma was determined: the total fibrinolytic activity (TFA), plasminogen activator activity (tPA) and XIIa-dependent fibrinolysis (XIIa). In the blood was measured glucose level. All experiments were complied with the Declaration of Helsinki.

Results: Within 1 h after the last injection PRG SFA increased by 32%, tPA - 105%, and XIIa - 92% compared with control. The level of glucose in the experiment was 2 times lower than in control (as normal). After 6 days of undo the PRG on the back of continued receipt of glucose in the experimental group indicators tPA and XIIa were higher ($P < 0.001$) control. No significant differences in the level of glucose in experimental and control rats.

Conclusion: The data obtained indicate that Regulatory peptide Pro-Arg-Gly has protective antidiabetes action challenging the development of T2DM, and the parameters of hemostasis it prevents the development of depression functions anticoagulation system due to the increase fibrinolytic background plasma.

Disclosure of Interest: None declared.

PO207-MON

Experimental validation of a stochastic multiscale model of fibrinolysisBannish B¹, Kolev K² and Longstaff C³¹Mathematics, University of Central Oklahoma, Edmond, USA;²Semmelweis University, Budapest, Hungary; ³National Institute for Biological Standards and Control, South Mimms, UK

Background: Modeling and simulation of fibrinolysis is complicated by the fibrous structure of fibrin and the heterogeneous solid-fluid reaction system. A multiscale model has been developed using a stochastic approach to track individual tissue-type plasminogen activator (tPA) molecules, suitable for low concentrations. Microscale results feed into the macroscale model to simulate full 3D clot lysis. The model replicated published results and we have extended the validation to data collected under carefully controlled conditions.

Aims: 1) Determine fiber diameters and estimate pore sizes in standardized clots and follow clot lysis over a range of reactant concentrations and fibrin architectures. 2) Compare results from the mathematical model, using the same starting parameters.

Methods: Morphometric analysis of fibrin structure was studied by scanning electron microscopy and the kinetics of fibrin lysis were followed turbidimetrically. At the microscale, detailed biochemistry (binding/unbinding of enzymes, plasminogen (Plg) activation to plasmin, fibrin degradation, exposure of new binding sites) was used to model transection of single fibers. Results were fed into the macroscale model to generate clot lysis curves, analyzed as turbidimetric data.

Results: The model reproduced lysis curves without systematic deviation. Using 50% or 95% lysis times over a range of tPA and Plg concentrations (0.3–3 nM and 0.2–1.5 μ M, respectively), most model and data results were within 10%. For example, in a 100 μ L clot of fiber diameters 57.5–195 nm and pore sizes from 0.8–2.7 μ m, with 0.75 μ M Plg, 50% lysis times for 0.3, 0.6, 1.2 nM tPA were 5475, 6315, 8730 s, respectively, whereas model results were 5292, 6468, 8526 s.

Conclusion: The model generated accurate fibrin lysis profiles over a range of conditions. Future work will involve plasma clots with more components. The model will be a valuable tool to understand the regulation of existing or novel thrombolytics in different fibrins.

Disclosure of Interest: None declared.

PO208-MON

Thrombolytic preparation longolytin from low fungus resolves superficial haematomas

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Background: Preparation longolytin is stable proteinase's complex, isolated from culture of low fungus *Arthrobotrys longa*. It reveals high fibrinolytic, thrombolytic, anticoagulant and antiaggregation properties both in intravenous administration, and external application on thrombi of superficial veins. These data give evidence longolytin is able to penetrate through superficial tissues and, probably, to resolve shallow hypodermic hematomas, often formed in different everyday and medicine situations.

Aims: The aim is to study to reveal is longolytin possible to resolve experimental hypodermic hematomas.

Methods: Hematomas was formed in rats by hypodermic injection of 0.05 mL⁻¹ own blood in 2 points of body: above brisket (pectoral) and above groin (lateral hematomas). 0.3% longolytin in glycerol with heparin (10 μ mL⁻¹) was applied on hematoma every day during 7 days. There were determined resolving speed in 3 groups of rats: active group with longolytin and 2 control groups- with heparin and glycerol.

Results: Anatomical peculiarities were probably caused different pictures of hematomas: pectoral ones represented blue-like spot of big size – 310–800 mm², in lateral area there were formed blue-like hematoma's spot of on skin much smaller size – 50–250 mm². Independently of animal groups speed of resolving was firstly defined by the size of hematoma: in pectoral area it was maximal – 79 $\pm$ 12 in active group, 72 $\pm$ 12 in heparin group, 69 $\pm$ 11 in glycerol group. There was far smaller speed in little lateral hematomas – correspondingly 27 $\pm$ 4, 13.2 $\pm$ 2.5, 13.4 $\pm$ 3 mm² day⁻¹. Another influencing factor was revealed only in small lateral hematomas: longolytin application accelerated resolving speed twice. Time of resolving was 2.6 days for longolytin and 3.4 days for heparin and glycerol groups.

Conclusion: Different anatomical areas form various hematomas, differed in size and biological properties Longolytin accelerates only profound small hematomas's resolving, did not influence on great flattened ones.

Disclosure of Interest: None declared.

PO209-MON

The antifibrinolytic effect of factor XIII strongly depends on clot compaction/retraction

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Background: Although our insight into the underlying mechanisms of the effect of factor XIII (FXIII) on fibrinolysis has improved considerably in the last few decades, in particular by the discovery that activated FXIII cross-links alpha-2-antiplasmin to fibrin, the topic remains a matter of debate in which the experimental design of the fibrinolysis assays seems to be critical.

Aims: To study the mechanisms of the antifibrinolytic effect of FXIII.

Methods: Citrated platelet-poor plasma was supplemented with FITC-labeled fibrinogen, calcium and tissue factor to induce clotting and varying concentrations of tissue plasminogen activator (t-PA) to induce lysis, both in the absence and presence of 1 mM 1,3-dimethyl-2-[2-oxo-propyl]thioimidazol chloride, an inhibitor of activated FXIII. Clots were incubated for 0.5 h at 37 °C and subsequently compacted by centrifugation, after which the incubation was continued for 2.5 h whilst shaking. Clot lysis was assessed by measuring the released fluorescence.

Results: Fifty percent clot lysis was obtained with 80 ± 23 ng mL⁻¹ t-PA (mean $\pm$ SD) in the absence of FXIII inhibitor and with 10.4 ± 1.9 ng mL⁻¹ t-PA in the presence of FXIII inhibitor, indicating that 7.7 times more t-PA was required for lysis of cross-linked clots than for lysis of non-cross-linked clots. When the mechanical compaction was omitted only 1.6 times more t-PA was required for lysis of cross-linked clots as compared to non-cross-linked clots. Assays with alpha-2-antiplasmin-depleted plasma showed no effect of cross-linking on clot lysis, indicating that the antifibrinolytic effects of FXIII in normal plasma clots are fully explained by cross-linking of alpha-2-antiplasmin to fibrin, both with and without compaction.

Conclusion: The antifibrinolytic effect of factor XIII strongly depends on clot compaction. The most likely explanation is that cross-linking of alpha-2-antiplasmin prevents that the inhibitor is expelled from the clot during compaction/retraction.

Disclosure of Interest: None declared.

PO210-MON

The association of PAI-1 and clot lysis time with body composition in Africans is sex dependent

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Background: Obesity has been shown to be strongly associated with impaired fibrinolysis, mainly through associations with plasminogen activator inhibitor type-1 (PAI-1). Much less is known regarding the relationship with fibrinolytic potential (clot lysis time).

Aims: We compared the associations of PAI-1 activity (PAI-1_{act}) and clot lysis time (CLT) with body composition in a group of black South African adults. Special attention was paid to the influence of different fat distribution patterns between sexes on PAI-1_{act} and CLT.

Methods: Data for 1 288 apparently healthy black South African men and women aged 35 and older were cross-sectionally analysed. Body composition data included: body mass index, waist circumference, waist to height ratio, skinfolds and percentage body fat as measured with air-displacement plethysmography (ADP) and bioelectrical impedance (BIA). This study complies with the ethical principles of the Helsinki declaration.

Results: Despite adjustment for differences in body composition, PAI-1_{act} levels and CLT were significantly higher in women than in

men. PAI-1_{act} had a stronger association with central obesity while CLT had a stronger association with total body fat. In women PAI-1_{act} and CLT associated differently with body composition markers. CLT showed a linear relationship with body composition markers where PAI-1_{act} levels plateaued at higher body composition categories and showed no association with skinfolds. In men PAI-1_{act} and CLT had similar, linear relationships with body composition markers.

Conclusion: Observed differences between men and women may be related to differences in adipose tissue type, distribution and sequence of accumulation. PAI-1_{act} is strongly influenced by accumulation of visceral adipose tissue whereas CLT is associated with total obesity independent of type and sequence of body fat accumulation. The association between CLT and body composition is, therefore, at least in part, independent of PAI-1.

Disclosure of Interest: None declared.

PO211-MON

Fibrinolytic dysregulation in arthroplasty patients may contribute to postoperative hemostatic complications

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Background: The pathogenesis of the alterations of fibrinolytic components in inflammatory joint disease and their post-surgical modulation are not clearly understood. Preexisting hemostatic dysfunction may lead to both thrombotic and bleeding disorders in these patients.

Aims: To profile fibrinolytic parameters in patients undergoing arthroplasty prior to and after surgery and compare with normal, healthy controls.

Methods: Hundred arthroplasty patients were included in this study. Blood samples were drawn at preoperative (preop) and postoperative (postop) day 1. Citrated plasma samples from 50 healthy individuals constituted the control group. Antigenic level of D-Dimer, PAI-1 and tPA were measured using a commercially available ELISA kit. Antiplasmin activity was measured by using functional method.

Results: Preop PAI-1, D-Dimer, tPA levels were significantly higher from healthy controls ($P < 0.0001$), ($P < 0.0001$), ($P < 0.0001$); respectively. Preop antiplasmin levels were lower than controls ($P = 0.045$). Postop levels of PAI-1 and D-Dimer were increased compared to preop values ($P = 0.023$), ($P < 0.0001$); respectively. Postop antiplasmin values were lower than preop levels ($P = 0.024$). Changes in tPA were not significant ($P = 0.115$). There was no correlation between preop PAI-1 and D-Dimer levels. Pre and postop % changes of each individual were also calculated for PAI-1, D-Dimer, tPA and antiplasmin. There were significant correlation between D-Dimer and PAI-1 ($P = 0.003$). Negative correlations between antiplasmin and D-Dimer ($P = 0.0013$) and antiplasmin and PAI-1 ($P = 0.023$). There was no correlation between tPA and PAI-1, D-Dimer and antiplasmin.

Conclusion: These results confirm the preexisting perturbation of the fibrinolytic system of patients undergoing arthroplasty. Surgical intervention may further enhance the observed changes. The alterations in the fibrinolytic system may lead to the observed hemostatic complications in these patients.

Disclosure of Interest: None declared.

PO212-MON

Pre and perioperative factors modulate fibrinolysis in arthroplasty patients

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Background: Arthroplastic Surgery (AS) patients are mostly elderly and have other complications such as increased body mass index (BMI) and glucose levels. These factors, type of AS and use of tranexamic acid (TA) contribute to modulation of fibrinolytic (FL) system.

Aims: To demonstrate the effect of age, gender, BMI, type of AS and TA treatment on FL parameters in AS patients.

Methods: Blood samples of 99 AS patients were drawn at preoperative (preop) and postoperative (postop) day 1. Antigenic level of D-Dimer (DD), PAI-1 and tPA were measured using an ELISA method. Antiplasmin (AP) activity was measured by using functional method. Age, gender, status for TA treatment (10 mg kg⁻¹), type of AS, hemoglobin (Hgb) value and BMI were collected from records.

Results: Sixty nine female, 30 male patients with age 65 ± 10 underwent Total Knee AS (TKA: n = 67), Total Hip AS (THA: n = 32). Of the 99 patients, 25 were administered with TA. Preop DD levels were correlated with ages (P = 0.006), but not PAI-1. A positive correlation between age and preop tPA levels (P = 0.02) and negative correlation between age and AP level (P = 0.009). Positive correlation between BMI and preop tPA levels (P = 0.015) was observed but not with PAI-1, DD and AP. The correlations for preop glucose level with PAI-1 and DD were not significant. There were no significant differences in postop levels of DD, PAI-1, tPA and AP between TA treated or not. In terms of % changes, the DD and tPA, showed significantly lower changes in patients treated with TA compared to non-treated group, respectively (P = 0.037), (P = 0.007); PC for PAI-1 and AP were not significantly different. Administration of TA did not change postop Hgb level. Postop levels of DD, PAI-1, tPA and AP weren't different between TKA and THA patients.

Conclusion: These results confirm the age is an essential component for contribution to FL dysregulation in addition to effect of BMI. TA treatment results in decreased FL activity in AS patients.

Disclosure of Interest: None declared.

PO213-MON

Kinetic and thrombolytic properties of Prourokinase-Pamam conjugates

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Background: Prourokinase (proUK) is plasminogen activator, which used as thrombolytic agent. The proUK disadvantages are rapid clearance from the blood and significant fibrinogenolysis. Dendrimers with specific size and shape and high content of functional groups on surface are new class of polymers as carriers for drugs.

Aims: Improvement of proUK properties by its modification with poly (amidoamine) (PAMAM) dendrimers.

Methods: Purified proUK was modified with activated PAMAM dendrimers of 2.5 and 3.5 generation in ratio 1:1, 1:5, 1:10 and 1:20 (M: M). The following properties proUK and proUK-PAMAM dendrimers were studied: stability of fibrinolytic activity in plasma; kinetics of Glu-Pg activation using conjugated method; thrombolytic activity by lysis of human plasma clots immersed in plasma.

Results: Optimal time for proUK conjugation with activated PAMAM2.5 and 3.5 was found to be 2 h. Degree of proUK modification

with PAMAM3.5 had little effect on its activation rate by plasmin and UK-PAMAM3.5 conjugates retained 95% amidase activity, while proUK-PAMAM2.5 conjugates were activated by plasmin slower and UK-PAMAM2.5 retained 85% activity. All conjugates activated Glu-Pg faster than free proUK: maximum activation rate was 160% for proUK-PAMAM2.5 and 180% proUK-PAMAM3.5 (in ratio 1:5). All conjugates proUK-PAMAM2.5 caused 90-95% clot lysis in 4 h, while conjugates proUK-PAMAM3.5 (in ratios from 1: 1 to 1:20), showed decrease in lysis from 97 to 71%. Conjugation with dendrimers increased stability of proUK fibrinolytic activity (t_{1/2}=48 min): t_{1/2} increased from 60 to 130 min for proUK-PAMAM2.5 and from 54 to 115 min for proUK-PAMAM3.5 at increasing the ratio of protein:polymer of from 1:1 to 1:20.

Conclusion: Conjugates proUK with various modification degrees of its amino groups with PAMAM G2.5 and G3.5 were obtained. Conjugates proUK-PAMAM2.5 and proUK-PAMAM3.5 had increased the plasminogen-activator activity, higher stability in human plasma and somewhat reduced thrombolytic activity compared with free proUK.

Disclosure of Interest: None declared.

PO214-MON

The influence of residual platelets on plasma PAI-1 assays

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Background: The alpha granules of platelets were traditionally thought to contain mostly latent plasminogen activator inhibitor-1 (PAI-1). More recent publications, however, suggest that they also contain active PAI-1. In addition, the effect of residual platelets in plasma on PAI-1 fractions has not been studied in detail.

Aims: To investigate the effect of residual platelets in plasma on PAI-1 assays by relating a marker of alpha granule release, beta thromboglobulin (BTG), to PAI-1 activity (PAI-1_{act}) and antigen (PAI-1_{ag}), tissue plasminogen activator (tPA)/PAI-1 complex as well as a functional marker of fibrinolysis, plasma clot lysis time (CLT).

Methods: Samples were obtained from an apparently healthy group of 75 black and 75 white South Africans and were centrifuged at 365 g for 10 min to obtain platelet containing plasma. The study complied with the Helsinki Declaration and informed consent was signed after ethical approval was obtained.

Results: BTG levels were significantly higher in blacks than in the whites (3264 [2009–4394] and 355 [218–584] IU mL⁻¹). In agreement with this, PAI-1_{ag} levels, comprising of latent PAI-1, active PAI-1 and tPA/PAI-1 complex, were significantly higher in the blacks (33.8 [28.4–42.4]) than in the whites (20.8 [16.7–25.8] ng mL⁻¹). No difference between the groups was found for PAI-1_{act}, tPA/PAI complex or clot lysis time, suggesting that the higher plasma PAI-1_{ag} is a result of higher latent PAI-1 levels. BTG correlated significantly with PAI-1_{ag} (r = 0.68, P < 0.0001) while only weakly with PAI-1_{act} (r = 0.22, P = 0.01) and CLT (r = 0.19, P = 0.02) and showed no correlation with tPA/PAI-1 complex.

Conclusion: The presence of residual platelets in plasma does not influence PAI-1_{act}, tPA/PAI-1 complex or clot lysis time significantly, but seems to be mainly related to an increase in latent PAI-1 plasma levels. These results suggest that plasma PAI-1_{ag} is more sensitive for the presence of platelets in plasma than PAI-1_{act}.

Disclosure of Interest: None declared.

PO215-MON

Clot lysis parameters are higher in inflammatory bowel disease patients with a history of thromboembolismBollen L¹, Castele NV¹, Peeters M¹, Vermeire S² and Gils A¹¹Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Laboratory for Therapeutic and Diagnostic Antibodies; ²Department of Gastroenterology, UZ Leuven, Translational Research in Gastrointestinal Disorders, Leuven, Belgium**Background:** Inflammatory Bowel Disease (IBD) is a chronic inflammatory disease of the gastrointestinal intestine, characterized by flares and remission. IBD patients are 3-fold more likely to develop thromboembolic events (TE) compared to the healthy population. During flares the risk rises up to 16-fold.**Aims:** To compare clot lysis parameters of IBD patients with TE (IBD+TE) to those of IBD patients without TE (IBD-TE), taking disease activity into account.**Methods:** Plasma samples of 118 IBD-TE (55% males, median [interquartile range] age of 37 [26 - 53] years) and 84 IBD+TE (45% males, median age of 50 [37–65] years) patients were collected. Three clot lysis parameters were determined by a functional clot lysis assay; the area under the curve expressed in optical density (OD) × minutes (AUC), the 50% clot lysis time expressed in minutes (50% CLT) and the amplitude expressed in OD. Disease activity was defined based on physician's global assessment.**Results:** The median time between plasma collection and TE event was 5 [1.8–11] years. IBD-TE patients had a median C-reactive protein (CRP) value of 3.7 [1.0–9.1] mg L⁻¹ and a mean platelet count of 303 [252–361] × 10⁹ L⁻¹ whereas IBD+TE patients had a median of 2.8 [0.8–8.2] mg L⁻¹ and 265 [198–333] × 10⁹ L⁻¹, respectively. At time of plasma collection, 36% of the IBD-TE patients and 25% of the IBD+TE patients had active disease. Comparing IBD-TE versus IBD+TE, the AUC (21.8 [12.8–31.3] vs. 31.2 [23.3–49.4], *P* < 0.005), the 50% CLT (95 [70–126] vs. 110 [84–134], *P* = 0.006) and the amplitude (0.241 [0.168–0.308] vs. 0.295 [0.222–0.436], *P* < 0.005) were significantly different and remained significantly higher in IBD+TE after adjustment for age, gender, CRP, type of disease, presence of comorbidities and disease activity.**Conclusion:** These results show that all three clot lysis parameters are higher in IBD patients with a history of TE compared to IBD patients without TE when corrected for confounding factors.**Disclosure of Interest:** None declared.

PO216-MON

Tissue plasminogen activator (t-PA) released from human cells by “Soft rush “ [*Juncus Effusus* L. Var. *Decipens*]Sumi H, Imai M² and Mizote H²Life Science, Kurashiki University of Science and the Arts, Kurashiki²OKISO Co., Ltd., Okayama, Japan**Background:** It has been known that there are several essential oils which promote blood coagulation and fibrinolytic activities (Sumi, *Aroma Res.*, 4:60, 2003; Sumi and Yatagai, CRC Press New York, p.185, 2007). We observed *Juncus effusus* (the main raw material for “tatami”) containing these activities on human cells.**Aims:** This study aimed to show the expression of t-PA remarkably and the same extract showing anti-platelet aggregation activity.**Methods:** Mud-dyed and pesticide-free “soft rush “ [*Juncus effusus* L. *decipens*] harvested in 2013 was extracted with ethanol. The culture solution of HeLa cells (Sumitomo Dainippon Pharma Co., Ltd.) obtained after subjecting to culture for 24 and 48 h was recovered and established as the test medium (1st and 2nd medium) (Yatagai *et al.*,*Pathophysiol. Haemost. Thromb.*, 36:227, 2009). Amidolysis measurement of t-PA, H-D-Ile-Pro-Arg-pNA was used and the activity was calculated (unit mL⁻¹) based on the amount of p-NA produced (Sumi *et al.*, 22nd ISFP, P36, 2014). The anti-platelet aggregation activity was carried out as reported previously (Sumi *et al.*, *Jpn. J. Alcohol & Drug Studies*, 46:297, 2011).**Results:** As a result, the addition of soft rush extract on human cells, the t-PA activity increased in the concentration-dependent manner and the maximum value was 20-fold compared to the control. As human cells, 1st and 2nd test medium were used, almost the similar tendency were observed. On the other hand, in the platelet aggregation test, the same ethanol extract was found to strongly inhibit ADP and collagen-induced aggregation activity.**Conclusion:** This research for the first time showed that human cells added with culm extract of *Juncus effusus* promoted high expression of t-PA. There is a current report that β-phenethyl alcohol which is the main component of rose oil increased release of t-PA (Sumi *et al.*, *Jpn. J. Alcohol & Drug Studies*, 47:75, 2012). Further research is needed in areas such as peace of mind and enhancement of learning and memory.**Disclosure of Interest:** None declared.

PO217-MON

Global fibrinolytic profile in patients with chronic thromboembolic pulmonary hypertensionLebreton A¹, Casini A², Lador F³, de Moerloose P² and Neerman-Arbez M⁴¹Hematology department, CHU Clermont-Ferrand, Clermont-Ferrand, France; ²Angiology and Haemostasis Department;³Pneumology department, University Hospital of Geneva;⁴Genetic Medicine and Development department, University medical centre, Geneva, Switzerland**Background:** Chronic thromboembolic pulmonary hypertension (CTEPH), a complication of pulmonary embolisms, is due to incomplete pulmonary reperfusion and a remodeling of the pulmonary vessels leading to an elevation of the pulmonary vascular resistance. Impaired fibrinolysis and residual fibrin persistence could be involved. Five fibrinogen mutants have been associated with CTEPH**Aims:** Our aim was to evaluate the correlation between fibrinogen genotype and resistance to fibrinolysis as measured by a global fibrinolysis assay in patients with CTEPH.**Methods:** Eight patients with CTEPH, 16 controls and two dysfibrinogenemic patients with known fibrinolysis resistance were included. The plasma fibrinolytic potential of a tissue-factor triggered clot at two concentrations of t-PA (400 ng mL⁻¹ or 200 ng mL⁻¹) was assessed by the clot lysis time (CTL), defined as the time needed when the maximum OD is reduced to its 50% level. All the exons and intron-exon junctions of the fibrinogen genes were amplified by polymerase chain reaction and sequenced.**Results:** The mean CLT was of 9.8 (7.5–12), 9.4 (7.5–11), 9.8 (7–13) and 15 (14–16) min with 400 ng mL⁻¹ of t-PA and of 33.3 (26–37), 31 (28–35.5), 29.6 (16.5–36) and 43 (39–47) min with 200 ng mL⁻¹ of t-PA in CTEPH patients, healthy, anticoagulated controls and dysfibrinogenemic patients, respectively. Two CTEPH patients presented a resistance to fibrinolysis at both t-PA concentrations compared to controls. None of the previously reported mutations associated with CTEPH were found in our patients. Analysis is ongoing to identify other causative mutations, if any. PAI and alpha-2 antiplasmin were in normal range for all patients.**Conclusion:** In this study, two patients with CTEPH presented an impaired fibrinolysis as defined by the CTL. Any fibrinogen mutant was identified, suggesting that the impaired fibrinolysis is not secondary to an abnormal structure of the fibrinogen/fibrin. A larger study is

needed to confirm if hypofibrinolysis could be involved in the development of CTEPH.

Disclosure of Interest: None declared.

PO218-MON

Measurement of *in vivo* generated carboxypeptidase U (CPU, CPB2, TAFIA) in patients with acute myocardial infarction

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Background: Two decades after its discovery, carboxypeptidase U (CPU, CPB2, TAFIA) has turned into an interesting drug target in thrombosis research. However, considering the difficulty of active CPU measurement in the blood circulation, previous clinical studies mainly focused on the measurement of its zymogen, proCPU.

Aims: Using a specific and sensitive enzymatic assay, we investigated whether we can detect active CPU levels in the blood circulation and whether CPU is increased in patients presenting with acute myocardial infarction (AMI).

Methods: Peripheral arterial blood samples were collected from 45 patients with AMI (25 STEMI, 20 NSTEMI) and 27 controls. Additionally, in 11 STEMI patients, we obtained intracoronary blood samples during thrombus aspiration. All samples were collected in trisodium citrate tubes containing PPACK and aprotinin, thereby avoiding any *ex vivo* activation of proCPU by thrombin or plasmin. CPU plasma concentrations in these samples were measured by means of an activity-based assay using Bz-o-cyano-Phe-Arg as substrate.

Results: CPU activity levels were significantly higher in patients with AMI in comparison to controls ($P = 0.0007$). No correlation between CPU levels and AMI type (NSTEMI vs. STEMI) was found ($P = 0.654$). Intracoronary samples resulted in significantly higher CPU levels than the peripheral samples, indicating local CPU generation ($P = 0.020$).

Conclusion: These data suggest the *in vivo* generation of functional CPU during the process of plaque rupture and subsequent thrombus formation in patients with acute myocardial infarction.

Disclosure of Interest: None declared.

PO219-MON

Characteristics of carboxypeptidase U (CPU, CPB2 OR TAFIA) generation during *in vitro* clot lysis

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Background: Carboxypeptidase U (CPU, TAFIA) has become an attractive novel drug target in the field of thrombotic diseases. Improved insight in the process of CPU generation is essential in view of the upcoming development of CPU inhibitors.

Aims: The aim of this study was to investigate and validate the time course of CPU generation in healthy individuals, and to clarify mechanisms that influence this process.

Methods: CPU generation was monitored during an *in vitro* clot lysis assay by means of an enzymatic assay that uses Bz-o-cyano-Phe-Arg as specific substrate. The time course of CPU generation was determined in plasma of 21 healthy volunteers. A computer algorithm was

developed to automatically plot and quantify clot lysis and CPU generation related parameters.

Results: In all individuals, the typical biphasic pattern of CPU generation was present, but marked interindividual differences, both in height and in area under the curve (AUC) of the first and second CPU activity peak, were present. This method was validated and we determined a reference range for the different CPU generation characteristics in healthy individuals. The experimental data also provide more insight in the mechanisms that influence the CPU generation. The *Thr325Ile* polymorphism clearly is an important regulator of the half-life of the first CPU activity peak. This relationship was not observed for the half-life of the second peak. Additionally, a strong correlation was observed between the AUC of the first CPU activity peak and the Δ CLT ($r = 0.66$, $R^2 = 0.44$, $P = 0.0011$) and between the AUC of the second CPU activity peak and height of the clot lysis curve ($r = 0.76$, $R^2 = 0.58$, $P < 0.0001$).

Conclusion: Our method offers a technique to determine the individual endogenous CPU potential. Additionally, this method can be used as a tool to study mechanisms that influence CPU generation and therefore could be an interesting tool to investigate the effect of CPU inhibitors in more detail.

Disclosure of Interest: None declared.

PO220-MON

Cell-Free DNA modulates clot structure and impairs fibrinolysis in sepsis

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Background: Sepsis is a devastating condition characterized by systemic activation of inflammatory and coagulation pathways in response to microbial infection. Activation of neutrophils with microbial stimuli results in the release of neutrophil extracellular traps (NETs) comprised of cell-free DNA (cfDNA) that not only serve to trap pathogens, but also provide a stimulus for clot formation. Furthermore, we have previously identified elevated cfDNA levels to be a predictor of ICU mortality in septic patients. Though several studies on the effects of cfDNA and coagulation have been performed, little is known about the effects of cfDNA on fibrinolysis.

Aims: We have sought to (a) investigate the relationship between elevated cfDNA levels and impaired fibrinolytic activity in sepsis, and; (b) determine the mechanisms by which cfDNA modulates fibrinolysis.

Methods: Clot formation/lysis assays were performed on plasma from healthy volunteers and septic patients, as well as purified components. Coagulation via the intrinsic pathway was initiated by addition of a 15 mM CaCl₂ solution containing 1 nM tPA with or without plasminogen/plasmin. Fibrinolytic activity was measured by observing plasma turbidity at 450 nm.

Results: We observed an increased rate of clot formation and a delay in clot lysis in septic patient plasmas. In plasmas containing 5 μ g mL⁻¹ or more cfDNA, there was a complete inhibition of clot lysis, even after 5 h. Fibrinolytic activity was restored with DNase treatment. This effect was recapitulated in normal plasmas supplemented with increasing concentrations of purified cfDNA. In addition, our studies revealed that while cfDNA does not impact tPA-mediated plasmin generation, increased concentrations of cfDNA impair plasmin-mediated degradation of purified fibrin clots.

Conclusion: Our studies are the first to suggest that cfDNA impairs fibrinolysis in sepsis by preventing plasmin-mediated cleavage of fibrin. Our studies identify cfDNA as a potential pro-fibrinolytic therapeutic target in sepsis treatment.

Disclosure of Interest: None declared.

PO221-MON

Identification of functional procarboxypeptidase U (PROCPU, TAFI) in cerebrospinal fluid and its enhancement in the acute phase of brain injury

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Background: Carboxypeptidase U (CPU, CPB2 or TAFIa) is a basic carboxypeptidase that plays a vital role in fibrinolysis. Previous research stated that its precursor proCPU is not detectable in cerebrospinal fluid (CSF) from patients with isolated head trauma and controls. However, nowadays we have the ability to measure proCPU in a more selective and sensitive way.

Aims: This study aimed to examine the presence of the proCPU in CSF from patients presenting with brain injury due to blunt trauma (TBI), subarachnoid hemorrhage (SAH) and controls.

Methods: By using an *in-house* HPLC-assisted assay, we analyzed proCPU levels in CSF samples of 11 TBI patients and 19 SAH patients that were collected over a time-course of 12 days. Individuals ($N = 11$) under suspicion of meningitis but wherein biochemical and microbiological tests showed no abnormalities, served as controls.

Results: Low baseline functional proCPU concentrations were present in CSF of all controls. We found mean proCPU levels of 3.9 ± 1.8 U L⁻¹ (in comparison to plasma levels of 993 ± 161 U L⁻¹). In TBI and SAH patients, a pronounced elevation of proCPU (ranging up to 100 U L⁻¹) was observed in the hyperacute phase (≤ 24 h after onset) of brain injury and these levels declined progressively until normalization. Significant increased ($P < 0.05$) proCPU levels were observed up to 72 h after onset in SAH and up to 24 h in TBI.

Conclusion: This finding of the presence of functional proCPU in CSF, and its enhancement in the acute phase of TBI and SAH, could be of interest for the better understanding of the hemostatic disturbances and modulation of the blood-brain-barrier, frequently observed in these conditions.

Disclosure of Interest: None declared.

PO222-MON

In-vitro imaging of platelet/fibrin clot formation and lysis in flow condition

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Background: Thrombolytic therapy is of proven and substantial benefit for patients with acute cerebral ischemia. But the efficacy of the recanalization is variable and efforts are made to improve thrombolytic agents. However, validated *in vitro* methods are required to assess the lytic efficacy of drugs on whole blood clots and under flow conditions.

Aims: To develop an original methodology to evaluate in real time clot formation and lysis in whole blood and under arterial flow rates.

Methods: To mimic clinical situation, we designed a two-step approach: (i) Recalcified whole citrated blood supplemented DIOC6-labeled platelets and Alexa fluor 647-conjugated human fibrinogen is recalcified and immediately perfused over collagen (Horm, Nycomed) and tissue factor (Innovin, Dade) coated flow chambers (Vena8 Fluo+, Cellix) at a shear rate of 1500 s⁻¹. Thrombus formation and growth is continuously recorded and after 4 min, successive pictures along the channel are taken. (ii) After focusing on large fibrin(ogen)-rich clots and a second infusion of citrated blood containing rhodamine 6G-labeled platelets, supplemented with r-tPA and treated or not with antiplatelet or anticoagulant drugs is performed at the same shear rate. Real time changes in mean fluorescence of the fibrin(ogen)-rich network as well as of the platelets associated to the clot are measured allowing to quantify fibrinolysis and platelet behavior.

Results: Data confirm that a therapeutic concentration of r-tPA (15 µg mL⁻¹) significantly decreases the fibrin content of the clots. However, they reveal that r-tPA does not prevent the accretion of new platelets on the remnant thrombus. Interestingly, hirudin potentiates the rate and extent of fibrinolysis without modifying platelet accumulation that is, in contrast, decreased by antiplatelet drugs.

Conclusion: Overall, this *in vitro* global method allows assessing thrombolysis efficacy.

Disclosure of Interest: None declared.

PO223-MON

PAI-1 regulates fibrin clot stability and lysis

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Background: Plasminogen activator inhibitor-1 (PAI-1) plays an important role in the regulation of fibrinolysis by inhibition of tissue-type (t-PA) plasminogen activator activity. The proteolytic degradation of fibrin clots is mediated by plasmin, resulting from t-PA activity. Increases of PAI-1 levels are associated with thrombotic diseases and PAI deficiency with bleeding tendency, but its contribution to the hemorrhagic or thrombotic risk is still unclear.

Aims: We investigated how PAI-1 modulates fibrin structure stability and lysis by determining the temporal evolution of fibrin nanostructure (1,2).

Methods: Serial dilutions of Tissue Factor (0.5 to 5pM TF) or t-PA (125 to 200 ng mL⁻¹) were used in the assay. PAI-1 depleted plasmas were spiked with serial dilutions (0 to 200 ng mL⁻¹) of amiloride or purified PAI-1. The plasmas, incubated with low TF concentration and t-PA were triggered by calcium. The number of protofibrils was measured in real time during 30 min at 37 °C on a prototype routine instrument. Nanostructure parameters were determined according to (2).

Results: Increasing TF accelerated fibrin formation and increased fibrin stability. Increasing t-PA decreased stability and accelerated clot lysis. PAI-1 did not modify fibrin formation whatever the concentrations. PAI increased fibrin stability, leading to a high reduction of clot lysis. It seems to be a potent modulator of clot stability in the assay of fibrin nanostructure, as it immediately inhibits the [t-PA-fibrin-plasminogen] complex, resulting in decreased generated plasmin.

Conclusion: Our results show that PAI-1 has a major role in regulating fibrin stability and lysis, which may contribute to enhance the thrombotic or hemorrhagic risk. The fibrin nanostructure temporal profiles could be a potential determinant of this risk.

(1) Dassi et al. ISTH 2015 (submitted), (2) Yeromonahos et al. Biophysical J. 2010, Arterioscler Thromb Vasc Bio. 2012

Disclosure of Interest: None declared.

PO224-MON

The effect of alpha-2 plasmin inhibitor ARG6TRP polymorphism on the risk of coronary artery disease

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Background: The secreted full-length α₂-plasmin inhibitor (Met-α₂-PI) consists of 464 amino acids. In the plasma it undergoes both N- and

C-terminal cleavages, which significantly modify its activities. The N-terminal cleavage by APCE (α_2 -antiplasmin cleaving enzyme) results in 12 amino acids shorter isoform with Asn at the N-terminus (Asn- α_2 -PI). The ratio of Met- α_2 -PI:Asn- α_2 -PI in the plasma is 30:70. Asn- α_2 -PI isoform is cross-linked more effectively to fibrin α -chain, than Met- α_2 -PI by activated FXIII, thereby the alteration of the ratio of N-terminal isoforms may influence the fibrinolytic resistance of fibrin clot. The Arg6Trp polymorphism in the α_2 -PI gene may exert such an effect; as APCE cleaves the Arg6 form 8-fold faster than the Trp6 form, the Arg allele is associated with higher Asn1- α_2 -PI levels.

Aims: The aim of the case-control study was to explore the effect of α_2 -PI Arg6Trp polymorphism on the risk of coronary artery disease (CAD).

Methods: Eight hundred and forty seven patients admitted for coronary angiography to investigate suspected CAD and 999 individuals representing the Hungarian population were enrolled in the study. Patients were classified according to the extent of coronary atherosclerosis (CAS) and history of myocardial infarction (MI). Patients without significant CAS and with no history of MI were considered as clinical controls. α_2 -PI Arg6Trp genotype was determined by RT-PCR using LightCycler® 480.

Results: TRP allele frequency in the population control group was 21.5% (31.8% heterozygote, 5.6% homozygote), which showed good agreement with published allele frequencies. The presence of Trp allele did not influence the risk of CAS or MI when patient groups were compared to the clinical control or population control groups. Homozygous Trp6 genotype conferred a decreased, but statistically not significant risk of MI (OR: 0.59; CI: 0.28-1.27; $P = 0.176$), and the result needs to be confirmed on larger patient population.

Conclusion: In our study, the Arg6Trp polymorphism had no effect on the risk of CAD.

Disclosure of Interest: None declared.

PO225-MON

Thrombi formed at low haematocrit are resistant to fibrinolysis via a factor XIIIa-MEDIATED mechanism

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Background: Red blood cells (RBCs) have been considered a relatively inert bystander in coagulation and thrombus formation, yet their sheer abundance in blood means they dominate the resulting clot.

Aims: To examine the effects of haematocrit (HCT) on coagulation parameters, clot firmness and resistance to fibrinolysis.

Methods: Whole blood was separated into plasma and RBC constituents by centrifugation. Samples were reconstituted with 35% platelet rich plasma and RBCs to produce 20%, 40% and 60% final HCT. Thrombus formation and dissolution were assessed using Thromboelastography (TEG) and the Chandler Loop Model.

Results: TEG revealed significantly shorter clot time, clot formation time and increase in the α -angle at 20% HCT compared to 60% HCT; indicative of faster clot formation at lower HCT. An increase in maximum clot firmness was detected at 20% HCT, consistent with enhanced mechanical stability. Chandler model thrombi formed with 20% HCT were significantly longer than those formed at 60% HCT ($P < 0.05$) and demonstrated increased resistance to lysis by both tPA ($P < 0.005$) and uPA ($P < 0.005$). Inclusion of a transglutaminase (TG) inhibitor, to inhibit factor XIIIa, significantly increased lysis of 20% HCT thrombi ($P < 0.001$). Similarly, the TG inhibitor increased lysis of 40% HCT thrombi ($P < 0.01$),

albeit to a lesser degree, but no change in lysis was observed at 60% HCT ($P = 0.113$). Interestingly, when the TG inhibitor was present all thrombi lyse at an equivalent rate, suggesting that inhibition of factor XIIIa overcomes the stabilizing effect of HCT on lysis.

Conclusion: HCT has a dramatic impact on coagulation parameters, with lower HCT enhancing clot formation, resulting in thrombi with increased firmness and resistance to fibrinolysis. Factor XIIIa helps to maintain thrombus integrity and stabilizes against fibrinolytic degradation at lower HCT, an effect that is lost at higher HCT. These observations may help explain the increased risk of thrombosis in anaemic patients.

Disclosure of Interest: None declared.

PO226-MON

Impaired fibrinolysis in Behçet syndrome

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Background: Behçet syndrome (BS) is a systemic vasculitis characterised by oral and genital ulcers and uveitis. In addition to this classical triad, BS may affect every organ and system. Venous involvement with both deep and superficial thrombosis is the most common vascular manifestation of BS. The pathogenesis of thrombosis in BS is not well known. Inflammation seems to be the main trigger of thrombosis in BS, however different mechanisms by which inflammation is able to affect endothelial factors, coagulation, and fibrinolytic systems might contribute to thrombosis.

Aims: Impaired fibrinolytic activity has long been recognized as an important pathophysiological factor for thrombotic complications in BS. The aim of this study is to investigate fibrinolytic system alterations in BS patients.

Methods: In 25 patients with BS, and in 75 control subjects matched for age and sex, two global assays for fibrinolysis were performed: 1- Clotting and Lysis assay to study clot formation, morphology and fibrinolysis (EuroCLOT analysis) (Carter 2007); 2- Clot Lysis Time (CLT) to study global fibrinolytic capacity (Lisman 2005). In addition, some fibrinolytic parameters were measured, by using commercially available ELISA: Thrombin activatable fibrinolysis inhibitor (TAFI), plasminogen activator inhibitor (PAI-1) and Tissue Plasminogen activator (t-PA).

Results: Median TAFI and PAI-1 levels were significantly higher in BS patients than in healthy subjects ($P < 0.001$ and $P = 0.03$ respectively). Median tPA levels were similar between patients and controls. CLT tended to be increased in BS patients with respect to the control group but this difference did not reach a statistical significance. Finally, no difference was found between the two groups in the parameters of EuroCLOT analysis.

Conclusion: An impaired fibrinolysis is detectable in BS patients with increased levels of fibrinolysis inhibitors, TAFI and PAI-1, and a trend to prolongation of CLT. These alterations may contribute to the pathophysiology of thrombosis in BS.

Disclosure of Interest: None declared.

PO227-MON

Variable resistance to plasminogen activator initiated fibrinolysis for intermediate-risk pulmonary embolismStubblefield W¹, Kline J¹ and Rondina M²¹*Emergency Medicine, Indiana University School of Medicine, Indianapolis;* ²*Internal Medicine, University of Utah, Salt Lake City, USA***Background:** Optimal dose of plasminogen activating fibrinolytic agents may depend upon host resistance to fibrinolysis.**Aims:** To test plasma from patients with intermediate risk pulmonary embolism (PE) for degree of resistance to fibrinolysis catalyzed by tissue plasminogen activator (tPA) and elucidate the role of plasma proteins.**Methods:** Citrated plasma from 76 patients with submassive PE prior to injection of study drug, was compared with plasma from 20 age and sex matched patients without PE, as negative controls. Clotting was induced using CaCl₂, tissue factor, and phospholipid. Lysis was induced using 60 ng mL⁻¹ tPA. Time to 50% clot lysis (CLT) was assessed by both thromboelastography (TEG) and CLT was examined as a univariate predictor of outcomes, and plasma proteins (fibrinogen, α_2 -antiplasmin, plasminogen, thrombin activatable fibrinolysis inhibitor (TAFI), plasminogen activator Inhibitor 1 (PAI-1), and D-dimer) were tested as predictors of CLT in a multivariate model.**Results:** Compared with disease-negative controls, patients with PE exhibited significantly longer mean CLT on TEG (+43 min, 95% CI 23 to 62 min). Patients with PE and a short CLT who were treated with tenecteplase had increased risk of major or clinically significant bleeding (odds ratio for CLT < 1st quartile (3,842 s) was 11.6 (95% CI = 1.6–104). The diagnostic accuracy of CLT from TEG for bleeding had an area under the ROC of 0.711 (95% CI = 0.526–0.892; those with long CLT had significantly worse exercise tolerance and lower psychometric test scores for quality of life at 3 months. A multivariate stepwise removal regression model selected for both PAI-1 and TAFI as predictive factors for prolonged CLT (adjusted R² = 0.530).**Conclusion:** The CLT from TEG predicted increased risk of bleeding and clinical failure with tenecteplase treatment for intermediate-risk PE. Plasmatic PAI-1 and TAFI were independent predictors of CLT.**Disclosure of Interest:** W. Stubblefield: None declared. J. Kline Shareholder of: CP Diagnostics, Grant/Research Support from: AHRQ, NIH, Genentech, Pfizer and Octapharma, Consultant for: Genetech, Stago Diagnostica, Janssen Pharmaceuticals, M. Rondina: None declared.

Hemophilia – Clinical I

PO228-MON

Carrier and prenatal diagnosis of Chinese haemophilia a families in one center from 2007 TO 2014

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*Department Of Laboratory Medicine, Ruijin Hospital Affiliated To Medical College Of Shanghai Jiao Tong University, Shanghai, China***Background:** Haemophilia A is the most common X-linked inherited bleeding disorder. The female relatives are at risk of being carriers with the potential of passing the disorder on to their sons. So detection of carrier women in HA families and subsequent antenatal diagnosis of carriers are important for these families.**Aims:** In this report, we will introduce the major methods of carrier and prenatal diagnosis for Chinese HA families in our center.**Methods:** APPT, PT, TT, Fg, FVIII:C, FIX:C and VWF:RCo were detected to make the phenotypic diagnosis of HA. LD-PCR and PCR were adopted for the screening of the intron 22 and intron 1 inversion respectively. FVIII coding and boundary sequences were analyzed by

PCR and direct DNA sequencing. 7 STR sites related to F8 gene were combined together to do linkage analysis to confirm the mutation origin. AccuCopy method was used to detect the copy number variations (CNVs) of the F8 gene to confirm deletions or duplications.

Results: From Sept 2007 to July 2014, we did genetic diagnosis for 821 HA families. Tests showed 301 families were caused by inversions, 278 were INV22 (33.9%) and 23 were INV1(2.8%). For the other inversions negative families, 315 were due to the point mutations including the splicing mutations (38.4%), 150 were found small deletions/insertions (18.3%), 32 were caused by large deletions(3.9%) and 8 were caused by duplications (0.9%). After all of the detections, 15 families were still found no mutations in the F8 gene. Combined with the linkage analysis, we did carrier diagnosis for 1324 females in these families and detected 838 carriers. For prenatal diagnosis, 189 fetuses were normal, 57 were female carriers and 91 were male patients.**Conclusion:** Based on the present methods, we could make carrier and prenatal diagnosis for about 98% HA families. We can reasonably suggest that intron 22 and 1 inversions screening, F8 gene sequencing and CNVs detection combined with the linkage analysis are available for carrier and prenatal diagnosis in Chinese HA families.**Disclosure of Interest:** None declared.

PO229-MON

Genetic alterations in hemophiliac patients with inhibitor in the region of Murcia, SpainGarcía-Candel F¹, Melero-Amor A¹, Marín-Atucha N² and Romecín P²¹*Hematología-Hemoterapia, Hospital Clínico Universitario Virgen De La Arrixaca;* ²*Fisiología Humana, Facultad de Medicina-Universidad de Murcia, El Palmar, Spain***Background:** The development of inhibitors against factor concentrate administered is the main complication related to the treatment of Hemophilia today. Its appearance is related, among others, with certain types of gene mutations affecting the F8/F9, being the main risk factor in Hemophilia A. In Hemophilia B while not clearly established the influence of the type of mutation. The large deletions, nonsense mutations and inversion of intron 22 are more likely to occur, while the missense type, small deletions and insertions have a low risk.**Aims:** To determine the incidence and characteristics associated inhibitors at different mutations in the F8 and F9 genes in our patients.**Methods:** In the Region of Murcia there are 80 patients with hemophilia, of which 65 correspond to Hemophila A and 15 to Hemophilia B. We studied our population (median of 26 years (range 2-76)) and identified patients with a history of inhibitor. The genetic study was performed by PCR.**Results:** Are shown in the following table.

Patients with inhibitors		
PHemophilia type	Mutation	Transient Inhibitor (Yes/No)
SHA	p.Pro2153Leu	Yes
SHA	Inv. intron 22	No
SHA	Inv. intron 22	Yes
SHA	Inv. intron 22	No
SHA	p.Trp393Stop	No
SHA	Complete deletion of exon 26	No
SHA	Inv. intron 22	No
SHA	p.Trp585Cys	Yes

*SHA: Severe Hemophilia A.

Conclusion: Most high response inhibitors are related to high risk mutations, but it was a case of transient inhibitor in a case of intron 22 inversion and two cases of high response inhibitor in two cases of mis-sense mutations.

Disclosure of Interest: None declared.

PO230-MON

A retrospective evaluation of bleeding episodes in patients with haemophilia A, B and Von Willebrand's disease

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Background: Despite the regular use of factor concentrates and prophylaxis, children with hemophilia A, B and von Willebrand's disease (VWD) still experience severe acute bleeding episodes.

Aims: To determine the frequency and the most common sites of bleeding episodes of our patients diagnosed with haemophilia A, B and VWD.

Methods: Medical records of 81 patients who admitted to hospital for acute bleeding episodes between November 2009 and November 2014 were reviewed retrospectively.

Results: The patients were aged between 1- 24 years. 50 (61.7%) were diagnosed with haemophilia A, 10 (12.3%) with haemophilia B, 21 (25.9%) with VWD. Three (6%) patients with haemophilia A were positive for inhibitor.

Patients with haemophilia A admitted to hospital for a total of 1400 episodes. Of these 1176 (83.7%) were hemarthrosis, 89 (6.4%) intramuscular hematoma, 54 (3.9%) oral mucosal bleeding, 50 (3.6%) epistaxis, 25 (1.8%) hematuria, 2 (0.2%) intracranial hemorrhage, 2 (0.2%) splenic hematoma, 1 (0.1%) perisplenic subcapsular hematoma and 1 (0.1%) intraabdominal hematoma.

Haemophilia B patients admitted for 197 episodes. 136 (69%) were hemarthrosis, 28 (14.2%) intramuscular hematoma, 20 (10.2%) epistaxis, 11 (5.6%) oral mucosal bleeding, 2 (1%) were hematuria.

Patients with VWD (10 female, 11 male) admitted for 265 episodes. 74 (27.9%) were epistaxis, 68 (25.7%) menorrhagia, 58 (21.9%) oral mucosal bleeding, 32 (12%) hemarthrosis, 22 (8.3%) hematuria, and 11 (4.2%) intramuscular hematoma.

Conclusion: Hemarthrosis is the most frequent bleeding in children with hemophilia but these children always have risk of life threatening bleedings such as intracranial hemorrhage. There is always need for an effective factor replacement therapy in these children to prevent complications.

Disclosure of Interest: None declared.

PO231-MON

Antibodies of high avidity is the hallmark of severe hemophilia A patients with FVIII inhibitors

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Background: The immune system induces the production of specific antibodies in response to alloantigens and after several exposures antibodies will generally bind more tightly to the target antigen. The avidity of antibodies against factor VIII (FVIII) has not been studied in patients with hemophilia A (HA).

Aims: This study aimed to evaluate the avidity of anti-FVIII antibodies in HA patients and its association with clinical aspects of the disease.

Methods: We measured the avidity of antibodies anti-FVIII in Brazilian HA patients positive ($n = 34$; 13 for severe HA and 21 for moderate HA) and negative ($n = 22$; 7 for severe HA and 15 for moderate HA) for FVIII inhibitors. Urea treated and untreated plasma samples were used in ELISA with immobilized plasma derived FVIII to assess the antibodies avidity. The reactions were revealed using specific anti-IgG, anti-IgG1 and anti-IgG4 in a biotin/peroxidase system. The study was conducted in Fundação Hemominas, Belo Horizonte, MG, Brazil. The recruitment of participants and the methodology of the study were approved by the Brazilian ethics committees.

Results: The results showed that all patients have antibodies anti-FVIII detectable in ELISA with higher OD for IgG4 of patients with inhibitors ($P < 0.01$). Primary data showed that avidity of IgG and IgG1 were independent of HA severity and Bethesda titer. Otherwise, IgG4 from severe HA patients with inhibitors exhibited high avidity ($P = 0.02$). Antibodies from patients with low Bethesda titers ($<5 \text{ mL}^{-1}$) exhibited slightly higher IgG4 avidity when compared to patients with high Bethesda titers (88.0% vs. 71.3% of avidity index; $P = 0.04$).

Conclusion: The clinical effects of anti-FVIII antibodies avidity in HA are still unknown and more studies will be necessary. Due to its association to HA severity and to the inhibitor titer this parameter could be useful to monitoring the regular treatment of patients and possible the success/failure of immune tolerance protocols. Financial support: Fundação Hemominas, Fapemig, CAPES and CNPq.

Disclosure of Interest: None declared.

PO232-MON

The role of large population-based databases in the evaluation of safety of bypassing agents in hemophilia patients with inhibitors

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Background: Assessing whether the use of bypassing agents in hemophilia patients with inhibitors increases the risk of thromboembolic events (TEs) is challenging due to the rarity of both hemophilia and the occurrence of TEs. Administrative databases of healthcare transactions may permit a study under such conditions and reflect the treatments and outcomes as they occur in clinical practice.

Aims: To assess the incidence of TEs following exposure to inhibitor bypassing agents in hemophilia patients with inhibitors using a Medicaid Analytic Extract (MAX) database.

Methods: Among patients enrolled in Medicaid (2000–2006, 49 states and District of Columbia), we identified males with a diagnosis of hemophilia A or B and a dispensing of either recombinant factor VIIa (rFVIIa) or activated prothrombin complex concentrate (aPCC). Patients were followed until death, disenrollment for > 3 months, or end of study period to measure exposure to bypassing agents and the occurrence of TEs. Exposure was assessed on an as-treated basis, and TEs identified through diagnosis codes. Clinical review of chronological listings of claims served to adjudicate the TEs.

Results: A cohort of 408 patients (mean age 11 years) contributed 1,254 person-years (PYs) of observation. There were 5514 aPCC and 8782 rFVIIa dispensings recorded, which corresponded to 405 aPCC-exposed and 420 rFVIIa-exposed PYs. Of 21 potential TEs identified, fewer than 11 were classified as probable cases on adjudication. There were 12.5 TEs (95% CI: 6.2–22.8) per 1000 PYs exposed to any bypassing agent, 17.3 TEs (95% CI: 7.7–34.0) per 1000 aPCC PYs and 9.5 TEs (95% CI: 3.2–22.6) per 1000 rFVIIa PYs.

Conclusion: The small number of TEs identified underscores the rarity of this outcome among hemophilia patients with inhibitors. The combination of large size and extensive representation of children make MAX database useful for studying rare conditions that are prevalent in children, such as hemophilia.

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PO233-MON

Efficacy of secondary prophylaxis on annual bleeding ratio (ABR) in adult patients with hemophilia: a single center observation study in Japan

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Background: It has been reported that prophylactic infusions of factor concentrates was very useful to suppress joint bleedings in adult persons with hemophilia. In Japan, secondary prophylaxis as well as primary prophylaxis has become widespread and many severe or moderate hemophilic patients receive the treatment regardless of age.

Aims: To determine whether secondary prophylaxis in adult patients with hemophilia is effective or not and clarify their appropriate trough levels of coagulant factor during the prophylaxis, infusion doses and intervals.

Methods: Patient diaries from persons undergoing home infusion treatment have been collected from 2002 to 2014. Infusion days, dose, reasons of infusion and joint bleeding sites in respective patients were identified with reference to their diaries. We also assessed coagulant factor trough levels in the respective patients' plasma.

Results: Thirty-one adult patients' (A, $n = 25$; B, $n = 6$) data was investigated among the persons who kept their diaries accurately. Only 6 patients (severe hemophilia B, $n = 3$; moderate hemophilia A, $n = 3$) had on-demand treatment. On-demand treatment was replaced to prophylaxis in 18 of the remainder during the observation period. A common prophylactic regimen was twice a week or every 3 days in both hemophilia A and B. All of them except for 4 who received once-weekly prophylaxis had factor trough levels of $>1\%$. We divided their data into on-demand-year group (DG) and prophylaxis-year group (PG). Median annual bleeding ratio (ABR) in DG was statistically higher than that in PG (15.5 vs. 2.9, $P = 0.011$). Median ABR in 16 patients whose treatment had changed to prophylaxis was significantly reduced compared to the previous on-demand treatment periods (from 20.8 to 3.6, $P < 0.001$). Furthermore, mean ABR even in once-weekly prophylaxis was reduced to nearly half in comparison with their on-demand treatment periods.

Conclusion: Secondary prophylaxis is effective to reduce ABR of hemophilic patients even though once-weekly prophylactic regimen is selected.

Disclosure of Interest: None declared.

PO234-MON

Impact of the intrinsic pathway in individual thrombin generation among hemophilia B patients

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Background: Hemophilia B, an inherited bleeding disorder, is caused by deficiency of coagulation factor IX (FIX). Global hemostasis assays

show potential to evaluate bleeding tendency and factor replacement therapy but studies targeting hemophilia B are rare.

Aims: To evaluate treatment response of high dose recombinant FIX (rFIX) and the role of platelets and corn trypsin inhibitor (CTI) in global assays in hemophilia B.

Methods: Ten moderate and severe hemophilia B patients with informed consent received 60-100 IU kg⁻¹ of rFIX after ≥ 72 h wash-out, approved by local EC. Recovery was evaluated 30 min after infusion both with one stage clotting and chromogenic assay. Thrombin generation (TG) was observed with calibrated automated thrombogram (CAT) in platelet-poor (PPP) and -rich plasma (PRP) (150×10^6 platelets mL⁻¹) with 1pM tissue factor (TF). Thromboelastometry (ROTEM) was assessed with INTEM, EXTEM and diluted (1/17000) TF reagents. Citrate (0.109 M) with and without CTI (50 μ g mL⁻¹) were compared for blood collection.

Results: TG parameters were severely and uniformly reduced in PPP at baseline, varying more in PRP. FIX recovery was evident and TG correlated with FIX levels both in PPP and PRP; in PRP better with chromogenic than clotting assay. CTI delayed and decreased peak thrombin, but individual sensitivity to CTI varied especially in PRP (no change vs. $>50\%$ decrease) both at baseline and recovery. CTI impaired correlation between TG and FIX in PPP and PRP. Baseline INTEM was within reference in 4/10 patients, but infusion shortened clotting time (CT) in all, whereas EXTEM remained normal also at recovery. Diluted TF-method detected the dose response. CTI did not affect EXTEM, but prolonged CT with diluted TF.

Conclusion: Global assays can be used in treatment evaluation in hemophilia B with careful validation. ROTEM standard reagents fail to detect FIX deficiency and EXTEM also the recovery. CTI impairs TG individually in PRP, suggesting early hemostatic role of the intrinsic pathway.

Disclosure of Interest: V. Jokela: None declared, A. Jouppila: None declared, R. Lassila Consultant for: Astra Zeneca, Bayer, Boehringer Ingelheim, CSL Behring, the Medicines Company, Novo Nordisk, Pfizer.

PO235-MON

Safety and efficacy of recombinant factor VIII FC fusion protein (RFVIII FC) for the prevention and treatment of bleeding in previously-treated adult and adolescent subjects with hemophilia A: interim analysis of the aspire study

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Background: The safety and efficacy of rFVIII FC for the prevention and treatment of bleeding episodes in adults and adolescents with severe hemophilia A were shown in the phase 3 A-LONG study. Subjects who completed the A-LONG study could enroll in the extension study, ASPIRE.

Aims: To report interim data for the prevention and treatment of bleeding episodes in adults and adolescents in ASPIRE.

Methods: ASPIRE included 4 groups: individualized prophylaxis (IP; 25-65 IU kg⁻¹ every 3-5 days, or 2x/ week [20-65 IU kg⁻¹ Day 1; 40-65 IU kg⁻¹ Day 4]), weekly prophylaxis (WP; 65 IU kg⁻¹ every 7

days), modified prophylaxis (MP; if optimal dosing could not be achieved with IP or WP), and episodic treatment (ET). Subjects could change groups at any time; thus, a subject could be represented in more than 1 group.

Results: In total, 140/150 (93.3%) A-LONG subjects who enrolled in ASPIRE remained on study as of the interim data cut (median time on ASPIRE, 80.9 week). No inhibitors were observed; adverse events were typical for a hemophilia A population. The median (interquartile range [IQR]) annualized bleeding rates (ABRs) in each group were: IP ($n = 108$), 0.66 (0.00, 2.63); WP ($n = 27$), 2.03 (0.60, 4.39); MP ($n = 17$), 1.97 (0.96, 7.03); ET ($n = 14$), 18.36 (10.45, 30.46). For subjects using IP, WP, and MP, 39%, 22%, and 24%, respectively, had no bleeding episodes with rFVIII-Fc prophylaxis. Overall, types and locations of bleeding episodes were as follows: 62% spontaneous, 37% traumatic, 1% unknown; and 67% joint, 25% muscle, 5% skin/mucosa, 2% internal, <1% unknown. In total, 91% of bleeding episodes resolved with 1 rFVIII-Fc injection (97% with ≤ 2 injections). The median (IQR) average dose/injection utilized for a bleed in each group was: IP, 46.9 IU kg⁻¹ (26.9, 51.5); WP, 33.2 IU kg⁻¹ (26.7, 50.9); MP, 34.2 IU kg⁻¹ (29.5, 39.0); ET, 26.9 IU kg⁻¹ (25.0, 31.3).

Conclusion: ASPIRE interim data confirm results from A-LONG, demonstrating effective control of bleeding and maintenance of low ABR with rFVIII-Fc prophylaxis.

Disclosure of Interest: S. Jackson Grant/Research Support from: Pfizer and Baxter, Consultant for: Baxter and Biogen Idec, D. Perry Grant/Research Support from: Biogen Idec, Consultant for: Biogen Idec, D. Quon Consultant for: Baxter, Bayer, and Octapharma, Speaker Bureau of: Baxter, Biogen Idec, Grifols, and Novo Nordisk, P. Chowdary Grant/Research Support from: CSL Behring, Novo Nordisk, and Pfizer, Consultant for: Biogen Idec, Baxter, Pfizer, CSL Behring, and Novo Nordisk, Speaker Bureau of: Biogen Idec, Baxter, Pfizer, CSL Behring, and Novo Nordisk, A. Shapiro Consultant for: Baxter, Novo Nordisk, and LFB Biotechnologies, I. Pabinger Grant/Research Support from: CSL Behring, Consultant for: CSL Behring, and Pfizer, Speaker Bureau of: Bayer, Pfizer, CSL Behring, and Biotest, E. Santagostino Grant/Research Support from: Novo Nordisk and Pfizer, Consultant for: Bayer, Pfizer, CSL Behring, Novo Nordisk, Grifols, and Roche, Speaker Bureau of: Bayer, Baxter, Pfizer, CSL Behring, Novo Nordisk, Biotest, Kedrion, Octapharma, and Grifols, X. Li Shareholder of: Biogen Idec, Employee of: Biogen Idec, D. Glazebrook Shareholder of: Biogen Idec, Employee of: Biogen Idec, G. Allen Shareholder of: Biogen Idec, Employee of: Biogen Idec.

PO236-MON

New antiviral treatment of HCV infected young haemophiliacs –expectations to cure hepatitis C

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Background: New possibilities to cure hepatitis C.

Aims: Aim of the study is the long term observation of hepatitis C infected haemophiliacs and the effectiveness of the antiviral therapy.

Methods: Materials: 16 hepatitis C infected young adults were observed in our study, 6 of them more than 15 years. We report about the treatment by different therapeutic regimen acc. to the current development of the HCV-treatment experience. All our 16 patients carried the HC-virus and are HIV negative. HCV genotype was Simmonds 1b/Okamoto II.

Therapy: 1. Interferon (3 million units 3 times weekly) (1993).

2. Interferon + ribavirin (800–1200 mg day⁻¹) (1997).

3. PegIntron 1.5 µg kg⁻¹ BW. once per week + ribavirin for 48 weeks (2003).

4. Triple combination therapy with protease inhibitor (2011).

5. Nucleotid analog polymerase inhibitor sofosbuvir in double combination therapy (2014).

Results: Two patients underwent liver transplant after unsuccessful treatment efforts.

Another patients treatment had to be stopped after 6 months (2). Two patients had 48 weeks of combination therapy (3), but had a relapse. Only one patient could be successfully treated with combination therapy (3). 0.4.: Triple combination therapy (PegIntron, ribavirin and protease inhibitors boceprevir and telaprevir) used since 2011 was not applied.

5.: New combination therapy with sofosbuvir*, a nucleotid analog polymerase inhibitor.

- Sofosbuvir + Ledipasvir (Harvoni 400 mg 90 mg⁻¹), 1 tablet daily for 12 weeks.

- Sofosbuvir (Sovaldi 400 mg) and Daclatasvir (Daclinsa 60 mg), 1 – 1 tabl. Daily for 12 weeks.

Early studies show a virological response rate up to 100% in HCV infected patients. Our patients had no measurable HCV-PCR-RNA under the treatment.

Conclusion: Since 2014 new treatments show an increasing effectivity against HCV infection which means pecially for genotype 1b a new hope for cure of HCV infection.

*Sofosbuvir: HCV-NS5B inhibitor Daclatasvir inhibits the virus RNA replication and HCV-NS5A inhibitor Ledipasvir.

Disclosure of Interest: None declared.

PO237-MON

Analysis of bleeding rates in pediatric subjects with target joints and severe hemophilia A receiving prophylaxis with recombinant factor viii fc fusion protein (RFVIII-Fc) in kids a-long

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Background: rFVIII-Fc was safe and efficacious for the control and prevention of bleeding in pediatric subjects with severe hemophilia A in the phase 3 Kids A-LONG study.

Aims: To assess bleeding frequency in Kids A-LONG subjects with target joints (major joint with ≥ 3 bleeding episodes in a 6-month period) at baseline.

Methods: Kids A-LONG subjects were previously treated males aged <12 y with severe hemophilia A (<1 IU dL⁻¹ [$<1\%$] endogenous FVIII). All subjects received rFVIII-Fc prophylaxis 2x/week (25 IU kg⁻¹ Day 1 and 50 IU kg⁻¹ Day 4 initially, with adjustment if needed). Pre- and on-study bleeding rates were assessed.

Results: Thirteen subjects in Kids A-LONG had ≥ 1 target joint at baseline and 56 did not (median [interquartile range (IQR)] age, 6.0 y [5.0, 8.0] and 5.0 y [4.0, 8.0], respectively). Pre-study episodic therapy tended to be more common in subjects with target joints than without target joints (23.1% vs. 7.1%; $P=NS$). 11 subjects had 1 target joint; 2 subjects each had 2 target joints. The median (IQR) estimated number of bleeding episodes in the 12 months prior to study and on-study annualized bleeding rate (ABR) for subjects with target joints were 8.0 (4.0, 11.0) and 0.0 (0.0, 5.8); those for subjects without target joints were 2.0 (1.0, 4.0) and 2.0 (0.0, 3.9). For subjects aged <6 y with target joints ($n = 6$), pre- and on-study median (IQR) ABR were 6.0 (1.0, 8.0) and 2.8 (0.0, 6.0), respectively; those for subjects aged 6 to <12 y with target joints ($n = 7$) were 11.0 (5.0, 15.0) and 0.0 (0.0, 5.8), respectively. Twelve of 13 subjects with target joints at baseline did not continue to meet the pre-specified definition for target joints while on rFVIII-Fc prophylaxis (7 subjects did not have target joint rebleeding on-study). No new target joints developed in any subjects.

Conclusion: For subjects with severe hemophilia A and target joints, rFVIII Fc prophylaxis resulted in lower bleeding rates vs. pre-study FVIII treatment. No new target joints developed in this study.

Disclosure of Interest: B. Nolan Grant/Research Support from: Biogen Idec, G. Young Consultant for: Novo Nordisk, Biogen Idec, Baxter, Kedrion, and Bayer, Speaker Bureau of: Novo Nordisk and Biogen Idec, S. Rangarajan Grant/Research Support from: Pfizer, Grifols, and Baxter, Consultant for: Grifols and Biogen Idec, M. Wang Consultant for: Novo Nordisk, Biogen Idec, Baxter, and CSL Behring, B. Kerlin Consultant for: Novo Nordisk and Bayer, Speaker Bureau of: Novo Nordisk, Bayer, and rEVO, A. Leung Grant/Research Support from: Biogen Idec, E. Tsao Shareholder of: Biogen Idec, Employee of: Biogen Idec, D. Glazebrook Shareholder of: Biogen Idec, Employee of: Biogen Idec, G. Pierce Shareholder of: Biogen Idec, Employee of: Former employee of Biogen Idec, G. Allen Shareholder of: Biogen Idec, Employee of: Biogen Idec.

PO238-MON

Burden of illness among persons with hemophilia B: direct and indirect costs

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Background: Hemophilia poses large economic burdens, but costs specific to Hemophilia B are rarely examined separately from those specific to Hemophilia A and thus poorly understood.

Aims: To evaluate direct and indirect costs among persons with Hemophilia B in the USA by severity and treatment patterns.

Methods: Between 2009 and 2014, the Hemophilia Utilization Group Studies Part Vb (HUGS Vb) collected observational data from ten Hemophilia Treatment Centers (HTCs). Adult patients or parents of children with hemophilia B completed an initial survey assessing socio-demographics, clinical characteristics, and treatment patterns. Over 2 years, work absenteeism, underemployment due to hemophilia, and unpaid hemophilia-related caregiver time were recorded every 3 months to estimate indirect costs, using the human capital approach. Direct costs were estimated using 1-year healthcare utilization and 2-year drug dispensing records. All costs were annualized and converted to 2014 US dollars.

Results: One twenty one of 148 participants who had drug dispensing and healthcare utilization records and at least one follow-up survey were included. Prophylaxis was used by 4.8% of 62 patients with mild or moderate hemophilia and by 54% of 59 severe patients. Total mean annual direct and indirect per-person costs were \$79 495 for mild/moderate, \$204 692 for severe, and \$140 542 for all patients combined. Severe patients had statistically significantly higher direct and indirect costs compared with mild/moderate patients. Prophylaxis compared to episodic treatment was associated with lower mean indirect costs within severity class (mild/moderate: \$922 vs. \$4090; severe: \$6093 vs. \$11 787), and higher mean factor costs [mild/moderate: \$143 062 vs. \$71 219; severe (*P*-value 0.0051): \$271 209 vs. \$103 568].

Conclusion: Hemophilia B is associated with large direct and indirect economic burdens, as factor cost drives the high total expenditures. Within all severities, prophylaxis lowers indirect costs but raises factor costs.

Disclosure of Interest: None declared.

PO239-MON

Treatment of bleeding with recombinant factor VIII Fc fusion protein in previously-treated pediatric subjects with hemophilia A in the phase 3 kids a-long study

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Background: In the phase 3 Kids A-LONG study, recombinant factor VIII Fc fusion protein (rFVIII Fc) was safe and efficacious for the control and prevention of bleeding episodes in previously-treated pediatric subjects with hemophilia A.

Aims: To describe in greater detail the efficacy of rFVIII Fc for the treatment of bleeding episodes in Kids A-LONG.

Methods: Subjects eligible for Kids A-LONG were previously-treated males aged <12 y with severe hemophilia A (<1 IU dL⁻¹ [*<1%*] endogenous FVIII) and no history of inhibitors. rFVIII Fc was administered prophylactically. For treatment of bleeding, the dose of rFVIII Fc was based on the subject's known pharmacokinetic information and type and severity of the bleeding event. Key outcomes included number of injections, dose/injection, total dose, and subjects'/physicians' assessment of response to treatment of bleeding.

Results: In total, 71 subjects enrolled in Kids A-LONG; of these, 69 were treated with rFVIII Fc. Eighty-six bleeding episodes were reported. The median (interquartile range [IQR]) annualized bleeding rate was 1.96 (0.00, 3.96); 81.4% of bleeding episodes were controlled with 1 rFVIII Fc injection (93.0% with ≤2 injections). Per bleeding episode, the median (range) dose/injection required for bleeding resolution was 49.69 IU kg⁻¹ (13.9, 91.3) and the median (range) total dose was 54.90 IU kg⁻¹ (13.9, 200.0). For bleeding episodes requiring >1 injection, the median (IQR) time between the first and second injections was 23.88 h (16.08, 25.10). Subjects rated 92.6% of first injections (89.4% of all injections) to treat a bleeding episode as producing an excellent or good response. Physicians' global assessment of response to rFVIII Fc treatment was excellent or effective for 100% of subjects at study end.

Conclusion: Bleeding episodes were rare with rFVIII Fc prophylaxis in Kids A-LONG. rFVIII Fc was safe and efficacious for the treatment of bleeding in previously-treated pediatric subjects with hemophilia A.

Disclosure of Interest: E. Chalmers Grant/Research Support from: Baxter and Novo Nordisk, Speaker Bureau of: Baxter and Novo Nordisk, G. Young Consultant for: Novo Nordisk, Biogen Idec, Baxter, and Kedrion, Speaker Bureau of: Novo Nordisk and Biogen Idec, J. Mahlangu Grant/Research Support from: Bayer, Biogen Idec, Novo Nordisk, and Inspiration Biopharmaceuticals, Consultant for: Amgen, Bayer, Novo Nordisk, and Pfizer, Speaker Bureau of: Bayer and Novo Nordisk, M. Recht Consultant for: Kedrion and Novo Nordisk, A. Geddis: None declared, J. Alamelu: None declared, G. Gambino Shareholder of: Biogen Idec, Employee of: Biogen Idec, G. Pierce Shareholder of: Biogen Idec, Employee of: Former employee of Biogen Idec, G. Allen Shareholder of: Biogen Idec, Employee of: Biogen Idec.

PO240-MON

Acute effects of exercise on specific and global coagulation parameters in severe hemophilia AZetterberg E¹, Olsson HE¹, Susanna L¹, Thorsson O² and Berntorp E¹¹*Translational Medicine*; ²*Unit of Clinical Physiology and Nuclear Medicine, Skane University hospital, Lund University, Malmö, Sweden*

Background: It has previously been shown that exercise increases the coagulation capacity of healthy subjects and in patients with mild and moderate hemophilia A significant increases in FVIII: C and VWF activity have been seen. However, there is no available data on how the global coagulation activity in patients with severe hemophilia A is affected by exercise.

Aims: To study the effect of maximal exercise on global coagulation and individual coagulation factors in patients with severe hemophilia A and healthy controls.

Methods: Four patients with severe hemophilia A and 5 age matched controls performed a maximal exercise test on a bicycle ergometer. Blood samples were taken at 5 time points: during rest, 10 min, 1 h and 4 h after exercise. Global coagulation tests (thrombelastography and thrombin generation) as well as levels of FVIII: C and VWF activity were measured in both patients and controls. Levels of additional pro- and anticoagulant factors were only measured in controls.

Results: In the control group, values of thrombelastography and thrombin generation parameters indicated an increased coagulation capacity. Furthermore there was an increased activity of all pro-coagulant factors. In patients, the results of thrombelastography and thrombin generation assay indicated a *decreased* coagulation capacity after exercise. As expected, no changes of FVIII: C were seen, but VWF: Ag and activity increased over time.

Conclusion: Our data demonstrated a *decreased* coagulation capacity in patients with severe hemophilia. However, in the patient group there was an increase in VWF: Ag and VWF activity over time. We speculate that this increase in VWF activity will prolong the half-life of factor VIII concentrate given *before* exercise, a hypothesis that will be addressed in coming studies.

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PO241-MON

First prospective results of joint distraction in haemophilic ankle arthropathyvan Vulpen LFD^{1,2}, Vogely HCh³, Roosendaal G¹, de Kleijn P¹, van Roermund PM³, Mastbergen SC², Lafeber FPJG² and Schutgens REG¹¹*Van Creveldkliniek*; ²*Rheumatology & Clinical Immunology*;³*Orthopaedic Surgery, University Medical Center Utrecht, Utrecht, Netherlands*

Background: In haemophilia nowadays most bleedings occur in the ankle, and joint fusion is often necessary already at a young age. Joint distraction, an effective treatment in ankle osteoarthritis, is an attractive alternative and preserves joint motion. Earlier, we reported good clinical and structural efficacy in a retrospective evaluation of 3 haemophilia patients.

Aims: To gather prospective data on clinical effectiveness and tissue structure changes of ankle joint distraction in haemophilic arthropathy.

Methods: Haemophilia patients (≥ 18 and < 45 years) with severe complaints of arthropathy in the tibiotalar joint, insufficiently responding

to conservative treatment, were eligible. Informed consent was obtained prior to inclusion and the study was approved by the local medical ethics committee. Joint distraction was performed for 10 weeks. Clinical effectiveness was evaluated via questionnaires and physical examination at baseline, 6 and 12 months. Functional tests, X-ray and MRI examination were performed at baseline and 1-year follow-up.

Results: In 3 patients, 12 months follow-up is available (age at time of surgery 22–33 years). Pain decreased from 65 (47–76)mm at baseline to 9 (7–84)mm and 12 (1–43)mm at 6 and 12 months follow-up. Self-perceived functionality, measured by the Haemophilia Activities List and Ankle Osteoarthritis Scale, improved in 2 patients at 6 months, and in all at 12 months. Functional tests improved in all patients at 1-year follow up. MRI revealed a clear decrease in volume of subchondral cysts and bone edema in all, and slight improvement of joint space width in one patient.

Conclusion: This first prospective study investigating the efficacy of joint distraction in haemophilic ankle arthropathy, showed clear clinical and structural improvement in all patients at 1 year follow-up. Although preliminary, these data indicate that joint distraction may be a promising treatment postponing more rigorous surgery like ankle arthrodesis in those patients not benefitting from conservative therapy.

Disclosure of Interest: None declared.

PO242-MON

Initial results of a clinical trial evaluating a full-length pegylated recombinant factor viii with extended half-life for the perioperative control of hemostasis in hemophilia ABrand B¹, Gruppo R², Wynn TT³, Griskevicius L⁴, Fernandez MFL⁵, Greblikas F⁶, Dvorak T⁷, Patrone L⁶, Fuerlinger M⁷ and Abbuehl BE⁷¹*Universitaetsspital Zuerich, Zuerich, Switzerland*; ²*Cincinnati Children's Hospital Medical Center, Cincinnati*;³*University of Florida, College of Medicine, Gainesville, USA*;⁴*Vilnius University Hospital Santariskiu Klinikos, Vilnius, Lithuania*;⁵*Complejo Hospitalario Universitario A Coruña, A Coruña, Spain*;⁶*Baxter Healthcare Corporation, Westlake Village, USA*; ⁷*Baxter Innovations GmbH, Vienna, Austria*

Background: BAX 855 is a PEGylated full-length recombinant factor VIII built on ADVATE with an extended half-life. BAX 855 demonstrated efficacy and safety in prophylaxis and the treatment of bleeding episodes in previously treated patients with severe hemophilia A. (Konkle et al. Haemophilia, 2015 EAHAD Congress)

Aims: This phase 3 surgery study is evaluating the efficacy and safety of BAX 855 for perioperative control of hemostasis.

Methods: Patients' informed consent and appropriate ethics committee approvals were obtained. Elective procedures were prospectively classified (major or minor) by the investigator/surgeon; major emergency surgeries were excluded. The dose and frequency of BAX 855 administered perioperatively was guided by each patient's pharmacokinetic profile for major procedures or BAX 855 incremental recovery for minor procedures. Subjects participated until discharge or upon resuming pre-study treatment regimens.

Results: To date, 15 male patients ranging from 19 to 52 years of age have undergone 15 procedures in 7 countries. Ten patients were naïve to BAX 855 and 5 had participated in the BAX 855 pivotal study. Of the procedures, 11 were classified as major and 4 were minor. Most (7) procedures were orthopedic (6 were major) and 4 were dental (3 were major). The remaining 4 were major cardiovascular, major abdominal, minor endoscopy, and minor dermatological procedures. The intraoperative efficacy of BAX 855 to provide hemostatic control was rated

by the operating surgeon as "excellent" for all 15 procedures. No factor VIII inhibitors have been reported and no new safety issues have been identified.

Conclusion: These preliminary results suggest that BAX 855 is safe and efficacious for the perioperative control of hemostasis in patients with severe hemophilia A. Results from the remaining procedures will confirm this profile.

Disclosure of Interest: B. Brand Grant/Research Support from: Baxter, R. Gruppo Grant/Research Support from: Baxter, Consultant for: Baxter, Pfizer, NovoNordisk, Speaker Bureau of: Alexion, T. Wynn Grant/Research Support from: Baxter, L. Griskevicius Grant/Research Support from: Baxter, M. F. Lopez Fernandez Grant/Research Support from: Baxter, F. Greblikas Shareholder of: Baxter, Employee of: Baxter, T. Dvorak Shareholder of: Baxter, Employee of: Baxter, L. Patrone Shareholder of: Baxter, Employee of: Baxter, M. Fuerlinger Shareholder of: Baxter, Employee of: Baxter, B. Abbuehl Shareholder of: Baxter, Employee of: Baxter.

PO243-MON

Evaluation of joint damage in boys with hemophilic arthropathy by ultrasonography and biomarker of cartilage turnover urinary C terminal telopeptide of type II collagen (U-CTX-II)

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Background: Arthropathy is a frequent and serious complication of repeated joint bleeding in patients with hemophilia, resulting in pain, deformity, and disability. Some biomarkers such as U-CTX-II appear to correlate with severity of joint degeneration.

Aims: The aim of the study was to evaluate joint damage in boys with hemophilia using ultrasonography (U/S), and to estimate biomarker of cartilage turnover U-CTX-II to determine its relation to the degree of hemophilic arthropathy (HA).

Methods: The study was carried out on Group I: thirty boys with hemophilia with a history of previous joint bleed, their age ranged from 6 to 15 years. Group II: Ten healthy children as controls with matched age and sex. An informed consent was obtained. The study was approved by the Local Medical Ethics Committee. Clinical assessment of the most affected or target joint using WFH clinical (Gilbert) score. U/S with concomitant radiological Pettersson scoring system to reveal the capacity of U/S in detecting osteochondral changes of HA. Estimation of U-CTX-II by ELISA.

Results: All patients were receiving on demand replacement therapy using plasma derived Factor concentrate or fresh frozen plasma according to availability. Fifteen patients (50%) had severe. Hemophilia, 7 (23.3%) had moderate and 8(26.72%) had mild hemophilia. U-CTX-II level was significantly higher in group I than in group II $P = 0.047$. There were positive correlations between Gilbert score with U/S score ($P = 0.005$) and U-CTX-II ($P = 0.001$). There was positive correlation between the U/S score and U-CTX-II ($P = 0.001$). There was no statistical significant difference between U/S and plain X-ray as regard osteochondral change.

Conclusions: U/S is non-invasive tool that helps in diagnosis of early stages of HA. U-CTX-II is a good indicator of the severity or even progression of joint damage in HA. It is associated with the burden of joint destruction; elevated level is highly correlated with radiographic progression.

Disclosure of Interest: None declared.

PO244-MON

Systematic monitoring of hemostatic management in hemophilia A patients with inhibitor in the perioperative period using rotational thromboelastometry

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Background: The management of hemophilia (H)A patients with inhibitors on bypassing therapy remains challenging. Accurate monitoring of these patients is particularly important in surgical situations. The recently developed comprehensive coagulation assays would be useful in these circumstances.

Aims: We have attempted to establish a systematic monitoring protocol using rotational thromboelastometry (ROTEM) to evaluate choice and effectiveness of different bypassing agents in the perioperative period.

Methods: The hemostatic effects of recombinant FVIIa (rFVIIa) and activated prothrombin complex concentrates (aPCC) were determined by ROTEM using a 3-step procedure (spike, preoperative, and perioperative) in eight patients with HA inhibitor (10–484 BU) admitted for elective surgery and assessed for individually tailored therapy. The ROTEM parameters, clot time (CT) and clot formation time (CFT), were evaluated in these assessments.

Results: In spike assessment, the ROTEM parameters demonstrated improvement to approximately normal levels with rFVIIa, although significantly shortened with aPCC in all cases. Pre-operative assessment was evaluated the effectiveness by infusion of each agent which doses were chosen by reference from spike date. The ROTEM parameters returned to normal levels in seven out of eight cases treated with rFVIIa, and five out of six cases with aPCC. In peri-operative assessment, patients were treated with appropriate agent and chosen dose according to the results of previous assessments, and ROTEM was performed immediately prior to each surgery. One case treated with aPCC and other seven cases treated with rFVIIa. In all cases, both of the parameters shortened enough after administration of the bypassing products and blood loss of surgery was minimal.

Conclusion: These findings demonstrated that this systematic analysis using ROTEM could provide a promising strategy for the use of bypassing therapy during surgery for HA patients with inhibitor.

Disclosure of Interest: None declared.

PO245-MON

Symptomatic female hemophilia seen in Taiwan

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Background: Symptomatic female hemophilia (H) is a rare and well documented disorder. No patients were reported in Taiwan before.

Aims: We report 3 patients of female HA and one patient of female HB. Informed consents were obtained.

Methods: HA and HB were diagnosed routinely. All exons and junctions of factor (F) VIII and FIX gene were amplified and sequenced. Multiplex ligation dependent probe amplification (MLPA) was used to detect large deletion in the FVIII gene. The methylation status of the

androgen receptor locus was examined to determine the X-chromosome inactivation (XCI) pattern.

Results: Patients I, II, III and IV were found to have severe HA (FVIII:C < 1%), moderate HA (FVIII:C 4.6%), severe HA (FVIII:C < 1%) and severe HB (FIX:C < 1%), at their age of 3,4 (year), 2 (day) and 25 (year), respectively. FVIII inhibitor was first detected in patient III at her age of 8. Cytogenetic analysis in patient III showed a chromosomal structural abnormality with 45X[22]/46X, idic (X) (q21) [8] Turner's mosaicism. Patient III has short stature, small breasts and underdeveloped genital organs, consistent with Turner's syndrome. A heterozygous Arg2228Gln transversion in FVIII gene, a heterozygous Ser1605Stop mutation in FVIII gene and a heterozygous Cys178Arg transversion in FIX gene were identified in patients I, II and IV, respectively. The mother of patient I was a carrier. MLPA analysis identified two large deletions of exon 1-22 and exon 1-26 of FVIII gene in patient III. Her mother had a heterozygous deletion of exon 1-22. A highly skewed ratio of 0:100 of XCI was noted in patients I and IV. XCI analysis was uninformative in patient II. A 50:50 ratio of XCI pattern was found in mothers of patients I, II and IV.

Conclusion: We report 2 female HA patients and one female HB patient, all 3 being identified as heterozygous carriers most likely with extreme lyonization, and one lady of severe HA who had chromosomal abnormalities associated with Turner's syndrome and a carrier mother.

Disclosure of Interest: None declared.

PO246-MON

No relation between platelet activity and hemophilia B phenotype

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Background: In hemophilia, unravelling the mechanisms behind inter-individual variability in bleeding tendency remains a big challenge. Hemophilia patients are classified according to their coagulation factor levels in mild, moderate and severe categories. However, coagulation factor levels do not always predict bleeding phenotype, as a substantial number of patients with severe hemophilia displays a mild phenotype.

Aims: Here we aimed to find parameters that contribute to individual hemostatic phenotypes in hemophilia B by comparing platelet activation and platelet responsiveness in hemophilia B patients to healthy controls and relating these to the clinical phenotype of the individual patients with severe hemophilia B.

Methods: Platelet reactivity was assessed in 12 healthy controls and 31 patients with hemophilia B (19 severe and 12 non-severe) through analysis of platelet P-selectin expression and α IIb β 3 activation after stimulation with platelet agonists. Plasma markers of systemic platelet activation were determined with ELISA.

Results: There was no difference in plasma levels of platelet release products β -thromboglobulin and RANTES or activation marker soluble P-selectin between controls and hemophilia patients, indicating there is no systemic platelet activation in hemophilia B. The response of platelets from hemophilia B to stimulation of the ADP-, thrombin-, collagen-, or thromboxane pathway did not differ from that of healthy controls. In the 19 patients with severe hemophilia, there was no relation between platelet activation or responsiveness and annual FIX consumption.

Conclusion: With the currently used platelet function analysis, we are not able to predict bleeding phenotype in hemophilia B.

Disclosure of Interest: None declared.

PO247-MON

Outcome of long term plasma derived FVIII concentrate Fanhdi[®] use in the mature haemophilia population

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Background: Since the introduction of coagulation factor concentrates, persons with haemophilia (PWH) have a life expectancy approaching the general population. The older PWH has to live with premature arthropathy and with HCV and/or HIV infection, but they also have age-related comorbidities such as cardiovascular, urological disease and cancer. Consequently, managing the older PWH is complex, and the long-term effect of coagulation concentrate on age-related health issues is unknown. Managing comorbidities such as ischaemic heart disease is challenging and continuing long term FVIII prophylaxis requires assessment of different issues to young PWH. Many older PWH registered at our centre have continued on a pdFVIII (Fanhdi).

Aims: To review the safety/efficacy of Fanhdi in the older PWH.

Methods: We performed a retrospective data analysis of PWH from St Thomas' Hospital, administered Fanhdi from 2003–2013.

Results: We included 49 patients (14 mild, 9 moderate, 27 severe) with Haemophilia A; median age of 49 (IQR 41–59) with the oldest PWH 93yo. 24 patients (21 severe) received prophylaxis (median Fanhdi dose of 3000iu kg⁻¹ per year) with median Annual Bleed Rate (ABR) of 1.3 and Haemophilia Joint Health Scores (HJHS) of 41.5. Only 4/27 PWH receiving on demand Fanhdi had calculable ABRs (range 11–38) with variable HJHS (13–40). Prior to 2003, 36 patients had HIV and 15 were co-infected with HCV. During the study period 7 patients developed cancer, 8 hypertension, 5 cardiovascular disease, 3 renal failure and 20 viral liver disease. There were no Fandhi-related reactions, inhibitors and no venous thrombotic events.

Conclusion: Long term Fanhdi maintained efficacy (ABR 1.3) even in the older patients with established arthropathy and co-infection. Over a 10 year period these patients had an incidence of cancer, hypertension and cardiovascular disease similar to aged matched population. There were no venous thrombotic events. Fanhdi had an excellent safety/tolerability profile.

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PO248-MON

Race and the immune response to factor VIII replacement therapy

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Background: The most common complication in hemophilia treatment is the development of alloimmune inhibitors which foreclose the ability of the infused factor (F) VIII to participate in blood coagulation. This occurs in approximately 25–30% of the hemophilia A (HA) population. These inhibitory antibodies confer significant pathology on the affected individual and present major complexities in their management. There are reports that inhibitors are more common in the black population, and it has been hypothesized that this phenomenon is a consequence of haplotype (H)-treatment product mismatch, since the recombinant products represent the FVIII encoded by H1 and H2.

Aims: To quantitate the haplotype-specific immune responsiveness of individuals treated for HA with FVIII replacement therapy.

Methods: Plasma samples from 488 HA subjects (222 black; 163 severe and 266 white; 178 severe) from 16 hemophilia treatment centers were analyzed for inhibitory and haplotype-specific α -FVIII antibodies. The inhibitor history (established by Bethesda assay) was provided by the treatment centers. The concentration of haplotype-specific antibody was quantitated by a multiplex immunoassay (detection limit = 7 nM) and 3 recombinant B-domainless FVIII proteins (H1/H2, H3, H4) produced in HEK cells.

Results: Seventy two black subjects (32%) and 68 white subjects (26%) had an inhibitor history. For severe HA subjects, the frequency was 36% and 25%, respectively. 94% black subjects and 91% white subjects had detectable α -FVIII antibody. In the majority of HA subject plasma samples, the total antibody was quantitated similarly for all B-domainless FVIII proteins.

Conclusion: For the entire study population, Fisher's 2-way test showed no significant difference between black and white HA subjects in the reported frequency of inhibitory α -FVIII antibody ($P = 0.11$). The α -FVIII total antibodies bound to H1/H2, H3 and H4 FVIII proteins similarly, independent of subjects' race and inhibitor status.

Disclosure of Interest: K. Mann Shareholder of: Haematologic Technologies, Consultant for: Baxter, Diagnostica Stago, Bayer, CSL Behring, Alnylam, S. Butenas: None declared, G. Pierce: None declared, R. Peters: None declared, S. Tan: None declared, J. Lusher: None declared, D. Lillicrap: None declared, C. Kempton: None declared, J. Mahlangu: None declared, A. Ameri: None declared, N. Key: None declared, R. Watts: None declared, A. Thompson: None declared, C. Leissinger: None declared, K. McRedmond: None declared, C. Walsh: None declared, C. Kessler: None declared, N. Josephson: None declared, D. Matthews: None declared, K. Pratt: None declared.

PO249-MON

Antibodies against polyethylene glycol (PEG) in healthy subjects – myth or reality?

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Background: PEG polymers are contained in a range of cosmetics and laxatives and used in several oral and parenteral applications in the pharmaceutical industry. The covalent attachment of PEG has emerged as successful strategy to increase the half-life of protein therapeutics. Several PEGylated FVIII- and FIX products are in development.

PEG has long been considered as non-immunogenic but recent reports have shown anti-PEG antibodies in healthy subjects and in some patients following treatment with PEGylated therapeutics. These reports have evoked controversial discussion due to limitations in the antibody analytics used.

Aims: To revisit the analysis of anti-PEG antibodies in healthy subjects using validated technologies.

Methods: Two cohorts of healthy human subjects ($n = 1310$) were analyzed for the prevalence of anti-PEG antibodies, applying separate calculations for antibody titers $<1:80$ and $\geq 1:80$. Two assay formats were used - a flow cytometry approach (FCM) and an ELISA platform validated with respect to cut-off calculation, sensitivity, specificity, linearity, and precision (inter-assay and intra-assay variability).

Results: Our data confirm the expression of anti-PEG antibodies by healthy individuals. The prevalence in our study cohort was 20–28%, with 12–26% for titers $<1:80$ and 2–11% for titers $\geq 1:80$. Age was identified as significant covariate for the expression of IgG antibodies, with the highest prevalence observed in young adults (18–30 years old). For the expression of IgM anti-PEG antibodies, age did not show significant influence. Competition experiments indicated that anti-PEG antibodies recognized PEG polymers of different sizes, e.g. linear PEG of 0.2, 2 and 20 kDa and branched PEG of 20 kDa. There was no indication of any pathology associated with these antibodies.

Conclusion: We confirm that some healthy individuals express antibodies against PEG. These antibodies might be induced by PEG polymers or their derivatives present in cosmetics or pharmaceuticals.

Disclosure of Interest: C. Lubich Employee of: Baxter Innovations GmbH, P. Allacher: None declared, A. Bauer Employee of: Baxter Innovations GmbH, T. Prenninger Employee of: Baxter Innovations GmbH, F. Horling Employee of: Baxter Innovations GmbH, J. Siekmann Employee of: Baxter Innovations GmbH, F. Scheifflinger Employee of: Baxter Innovations GmbH, B. Reipert Employee of: Baxter Innovations GmbH.

PO250-MON

Correlation between ABR and PK parameters in patients with severe haemophilia A receiving Turoctocog alfa (NOVOEIGHT®) for prophylaxis

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Background: Clinical severity of haemophilia A is closely related to FVIII:C levels with a significant inter-patient variability in bleeding phenotypes and pharmacokinetic (PK) parameters.

Aims: The aim of the present analysis was to examine the relationship between FVIII PK (half-life [$t_{1/2}$] and area-under-the-curve [AUC]) and bleeding pattern, i.e. annualised bleeding rates (ABRs) in a relatively homogenous group of patients with severe haemophilia A receiving routine prophylaxis with turoctocog alfa (Novo-Eight®).

Methods: This study analysed data from 60 patients with severe haemophilia A having a median age of 17.5 years (range 1.0–54.9 years) and a mean (SD) age of 17.0 (12.4) years. Thirty two (32) of the PK patients came from the guardian 3 trial (paediatric patients) and 28 patients came from the guardian 1 trial (adolescent and adult patients). Patients could also have participated in one of the PK trials (3522, 3600 and 2893) and could therefore have up to two PK sessions. In that case the mean PK value for each patient was used in the analysis. Turoctocog alfa PK was measured using one-stage clotting assay and chromogenic assay.

Results: There was no correlation between ABRs and PK parameters in the patient population studied. For the one-stage clotting assay, the Pearson correlation coefficients (r) were -0.034 for ABR versus AUC_{∞} and -0.052 for ABR versus $t_{1/2}$ and the P -values were 0.80 and 0.70, respectively. For the chromogenic assay, the Pearson correlation coefficients (r) were -0.012 for ABR versus AUC_{∞} and -0.026 for ABR versus $t_{1/2}$ and the P -values were 0.93 and 0.84, respectively.

Conclusion: Neither AUC nor $t_{1/2}$ correlates with bleeding pattern during routine prophylaxis with turoctocog alfa. Other factors such as individual compliance, joint status and physical activity need to be taken into account when optimizing a prophylactic treatment regimen.

Disclosure of Interest: V. Jiménez-Yuste Grant/Research Support from: Novo Nordisk, Pfizer, Grifols, Bayer, Baxter, Octapharma, Sobi and GSK, Consultant for: Novo Nordisk, Pfizer, Grifols, Bayer, Baxter, Octapharma, Sobi and GSK, S. Lejniec: None declared, A. Rosholm Employee of: Novo Nordisk A/S, N. Tripkovic Employee of: Novo Nordisk, E. Santagostino Grant/Research Support from: Pfizer, Bayer, Baxter, Novo Nordisk, CSL Behring, Grifols, Biotest, Consultant for: Pfizer

PO251-MON

Expression of platelet protein disulfide isomerase (PDI) is increased in patients with hemophilia or von Willebrand disease

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Background: Extracellular PDI, an abundant oxidoreductase with chaperone activity that is also present in human platelets, is critical to thrombus formation. In particular, PDI has been implicated in tissue factor activation, integrin regulation, and platelet-associated thrombin generation. Furthermore, impaired PDI release has most recently been shown to contribute to the bleeding tendency of Hermansky-Pudlak syndrome, an inherited platelet function defect.

Aims: To investigate platelet PDI expression in patients with congenital hemophilia or von Willebrand disease (VWD).

Methods: This study included patients with hemophilia A ($n = 24$) or B ($n = 4$), VWD ($n = 13$), or an unexplained bleeding disorder ($n = 10$) as well as healthy controls ($n = 24$). PDI expression was assessed by single-color flow cytometry on both resting platelets and platelets stimulated by either ADP or TRAP-6. CD62P (P-selectin) served as a positive control for α -granule secretion. The study protocol was approved by the local ethics committee and all patients and controls provided informed consent.

Results: Compared to healthy controls ($n = 24$), patients ($n = 51$) showed significantly increased PDI expression on both ADP- (median positive platelets, 3.6% vs. 1.3%, $P < 0.01$) and TRAP-6-stimulated

platelets (3.7% vs. 2.3%, $P < 0.01$), while baseline PDI expression was similar in the two groups (1.3% vs. 0.9%, $P = 0.1$). Importantly, this finding could not be explained by decreased agonist-induced platelet activation in the patient cohort. Furthermore, the pattern of increased PDI expression was consistent throughout the various study subgroups.

Conclusion: In this exploratory single-center cohort study, agonist-induced platelet PDI expression was significantly increased in patients with congenital hemophilia A or B, VWD, or a hitherto unexplained bleeding disorder. Up-regulation of platelet PDI could thus represent a compensatory mechanism under conditions of defective fibrin formation and/or platelet deposition.

Disclosure of Interest: M. Voigtländer: None declared, K. Holstein Grant/Research Support from: CSL Behring, Consultant for: CSL Behring, C. Dicke: None declared, B. Spath: None declared, C. Bokemeyer: None declared, F. Langer Grant/Research Support from: CSL Behring, Consultant for: CSL Behring.

PO252-MON

Alternations of oxidative stress markers in patients with severe haemophilia treated by different prophylaxis regimens

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Background: Due to recurrent bleeding in haemophilia patients, the effect of blood, i.e. free iron in the joint spaces, could initiate releasing markers of oxidative stress in physiological fluids.

Aims: The aim of this study were to estimate the influence of different prophylaxis regimens for severe haemophilia on alternations of oxidative stress markers in serum: a) advanced oxidation protein products (AOPP), b) paraoxonase-1 (PON1) and c) sulfhydryl groups (SHG).

Methods: The study included 20 adult patients with severe haemophilia. Five patients with haemophilia A received prophylaxis with FVIII concentrate in the standard dose of 20 IU kg⁻¹ three times per week, while another five patients with haemophilia A were given an intermediate dose of FVIII - 10–15 IU kg⁻¹ thrice weekly. Seven patients with haemophilia A and three with haemophilia B, received FVIII/IX concentrate only on-demand. The following oxidative stress markers were measured: a) AOPP, b) PON1 and c) SHG. Serum samples were collected initially, before the start of treatment (labelled AOPP-1, PON1-1 and SHG-1) and after 3 months follow-up (labelled AOPP-2, PON1-2 and SHG-2).

Results: The mean age of the patients was 32 years (range 19–55). In the group of patients given standard dose prophylaxis, the mean values of AOPP-2 ($P = 0.018$), PON1-2 ($P = 0.043$) and SHG-2 ($P = 0.045$) were significantly lower than those for AOPP-1, PON1-1 and SHG-1. Likewise, the mean values for AOPP-2 ($P = 0.047$) and PON1-2 ($P = 0.039$) in the five patients receiving intermediate dose prophylaxis were also decreased when compared to initial values, but SHG level was not significantly changed. In patients treated on demand the mean values for AOPP, PON1 and SHG did not alter significantly.

Conclusion: The results of this investigation showed that treatment of patients with severe haemophilia by continuous prophylaxis with intravenously applied FVIII/IX provides less oxidative stress reflected as decreasing of markers such as AOPP, PON1 and SHG.

Disclosure of Interest: None declared.

PO253-MON

Health-related quality of life in patients with hemophilia A and B in Brazil

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Background: Several studies show the negative impact of hemophilia in health-related quality of life (HRQOL) of patients. This issue is not well explored in developing countries.

Aims: This cross-sectional study aimed to evaluate the HRQOL and its associated factors in patients (≥18 years) with hemophilia A/B in Fundação Hemominas, a reference center for the hemophilia care in Minas Gerais, Brazil.

Methods: The study was approved by the Ethics Committee and all participants signed an informed consent. Data were collected by questionnaire and in medical records, including a Portuguese version of Haem-a-QoL. Statistical analysis were performed by ordinal logistic regression (proportional odds model) for each HRQOL domain (score divided into tercis).

Results: Patients attended during the period of August/2011 and July/2012 were invited to the study and 175 participants (147 with hemophilia A and 28 with hemophilia B) were included. The age of participants ranged between 18 and 68 years old and they were predominantly non-white (61.9%), unmarried (59.4%) and with more than 9 years of education (81.9%). The total score of the Haem-A-QoL had a median of 36.96 (range of 0–100), with worse performance in “sport and leisure” and best on “relationships” fields. HRQOL was worst among the older participants, the less educated, non-white, non-working, who were hospitalized in the last year, who did not have a single medical consultation and among those with the highest number of affected joints. Moreover, patients with hepatitis B had a significantly worse HRQOL in the domain “sports and leisure”, also observed in married patients. Otherwise, married individuals reported better HRQOL on “dealing with the disease”. Patients with hemophilia B reported worse HRQOL in the domain “Self perception”.

Conclusion: The results obtained will be worked and will allow the development of specific programs for the most vulnerable groups, improving the quality of life of hemophilia patients. This study was supported by FAPEMIG.

Disclosure of Interest: None declared.

PO254-MON

Comparison of prophylaxis and episodic treatment in children with moderate to severe hemophilia A

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Background: The aim of hemophilia treatment is to maintain patient's quality of life by preventing bleeding episode and joint damage. Prophylaxis treatment is currently being practiced worldwide. However, in Thailand most hemophilia patients were managed by episodic treatment.

Aims: This study determines to compare the efficacy of prophylaxis and episodic treatment in moderate to severe hemophilia A children at Chulalongkorn Hospital.

Methods: A prospective cohort study. Fifteen patients were included in this study from December 2012 to January 2014. Twelve patients were severe hemophilia A and three were moderate. Median age was 11.2 ± 5.3 years. All patients were treated with episodic standard guideline for six months, stopped for 1 month and went on prophylaxis treatment for another 6 months with infusion of 30–35 units kg⁻¹ of pd FVIII once a week. Evaluations included the number of bleeding episodes, admission days, school days lost and pdFVIII

used. Level of FVIII and FVIII inhibitor, joint score and quality of life score were also monitored.

Results: The number of bleeding episodes and school days lost in prophylaxis treatment were significantly lower than episodic (median 1 vs. 6 episodes and median 3 vs. 8.5 days, $P < 0.001$, respectively). Total number of pdFVIII used in prophylaxis treatment was higher than episodic (median 34.5 vs. 7.5 vial (500units), $P < 0.001$), but the number of pdFVIII replacement during bleeding episodes in prophylaxis treatment was less than episodic (median 1 vs. 7.5 vial (500 units), $P < 0.001$). Improvement of joint score during prophylaxis treatment was better than episodic (mean 1.58 vs. 1.33, $P = 0.846$). Quality of life score after prophylaxis treatment was significantly better than after episodic (median 85.7 vs. 72.9 $P = 0.002$).

Conclusion: Patients on prophylaxis treatment had better clinical outcomes including less bleeding episodes, less school days lost and better quality of life compared to episodic treatment.

Disclosure of Interest: None declared.

PO255-MON

Using minute doses of APTT reagent and tissue factor as the coagulation trigger to quantify the coagulation profile in hemophilia A

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Background: Large heterogeneity in bleeding patterns is observed in hemophilia A (HA). This may be attributed to that the available monitoring methods cannot echo the overall coagulation state in vivo; for instance, the APTT assay acts only through the intrinsic pathway, while the tissue factor (TF)-derived thrombin generation test (TGT) importantly depends on activation of the extrinsic pathway.

Aims: To set up global methods that determine the coagulation profile in HA, by modifying the coagulation trigger in TGT and ROTEM.

Methods: TGT and ROTEM were run in FVIII-depleted plasma spiked with rFVIII at different concentrations, and also in 9 patients with severe or moderate HA. The key components in coagulation trigger were APTT reagent (0.003% of the dose used in APTT method) and TF (0.02pM).

Results: In the FVIII-depleted plasma spiked with rFVIII (in vitro samples), different parameters detected by TGT and ROTEM varied in agreement with the doses of rFVIII added. This correlation to FVIII levels became undetectable when the in vitro samples was pre-incubated with prothrombotic proteins i.e. antibody (ab) to TFPI, ab to protein S, FEIBA or rFVIIa. In the patients with HA, the correlation to FVIII levels still existed; though its statistical significance was lower than that in the in vitro samples without the pre-incubation.

Conclusion: Decrease of the coagulation profile in HA is of course mostly due to low FVIII; other prothrombotic proteins also influence thrombin generation and fibrin formation, causing an imbalance between bleeding patterns and FVIII quantity. This study suggests that using minute doses of APTT reagent and TF to trigger coagulation in TGT or ROTEM may assess the whole coagulation picture behind the phenotypic heterogeneity in HA.

Disclosure of Interest: None declared.

PO256-MON

User experience assessment of the therapy management system Haemoassist® 2

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Hannover, Germany; ⁸Pfizer UK, Walton Oaks, UK

Background: The retrospective review of patients' paper diaries has been the gold standard for evaluating patient reported haemophilia care. Haemoassist® 2, a CE-marked medical device, combines a smartphone app that documents a patient's bleeds and factor usage with a physician website for real-time therapy analysis. Haemoassist® 2 evolved from Haemoassist® (Mondorf, Pollmann et al. *Haemophilia* (2009), 15, 464–472) whereby the respective legal manufacturer is StatConsult.

Aims: To guide the further development of Haemoassist® 2 by gathering structured user experience data and to facilitate data export into haemophilia registries.

Methods: The system's requirement specifications were developed with physicians, patients, legal, regulatory and medical device experts. A structured online user survey (9 domains, 25 items, 5-point scale) directed to German clinicians and nurses was conducted to assess user experience and guide the further development. Following up on earlier user requests, an export function to the German Haemophilia Registry (DHR) was developed with Paul-Ehrlich-Institute.

Results: Respondents (response rate: 87.5%, $n = 8$) indicated that Haemoassist® 2 can: help to detect target joints early (57.1% fully agree (FA), 42.9% rather agree (RA)), aid physicians to react faster to potentially therapy modifying events (71.4%FA, 28.6%RA) and may improve patient documentation compliance compared to a paper diary (42.9%FA, 42.9%RA, 14.3% rather disagree). Respondents were likely to recommend Haemoassist® 2 to a colleague (83.3%FA, 16.7%RA) and rated the system overall "(very) good" (8–9/10). Facilitated data entry to DHR was achieved developing and validating an *Export Client* which can be adjusted to other haemophilia registries.

Conclusion: The survey showed a high degree of satisfaction with Haemoassist® 2 underscoring the tool's potential to enhance patient safety and haemophilia therapy management. Haemoassist® 2 offers a flexible export function for haemophilia registries.

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PO257-MON

Once weekly Nonacog Alfa in hemophilia B (hemb): a time course profile of spontaneous bleeds in patients from a pivotal trial

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Background: HemB prophylaxis (PPX) varies from multiple to single injections per week. A recent pivotal multicenter, open-label study, in

moderately severe –severe patients (pts) (FIX:C ≤ 2) with PPX 1/week, 100 IU kg⁻¹ Nonacog showed a low median spontaneous (spont) annualized bleed rate (sABR)=1.0.

Aims: The time course of all spont & spont target jt (sTJ) bleeding after 1/week Nonacog dosing was examined to determine a pattern of bleeding day interval after PPX dosing & the extent that Nonacog PPX affected sABR & sTJ ABR.

Methods: Study report data were extracted for *post hoc* analysis. For each pt with ≥ 1 spont bleeding episode during PPX, the days from infusion to spont bleed & percent reduction from on demand (OD) to PPX in sABR & sTJ sABR were calculated.

Results: During PPX, 12 pts (48%) did not bleed & 13 (52%) had ≥ 1 spont bleeding episode.

No spont bleeds occurred on the day of dosing. The fewest bleeds occurred during day intervals 1- <2D (5%), ≥ 7 (8%) and 3 - <4D (9%) & 6 - <7D (11%). The highest percentage of spont bleeds occurred during day (D) intervals 5 - <6D (23%), 2- <3D, & 4 - <5D (22% both).

Reductions were observed both in any spont bleeding & sTJ bleeding, OD vs. PPX, in all 13 pts with ≥ 1 spont bleeding episode during PPX. sTJ ABR was 0.0 during PPX for 46% of these pts (6/13). Of these 13 pts, 92.3% (12/13) experienced sTJ ABR reductions $\geq 66.3\%$. Reductions in sABR ranged from 66.1% (27.2 $\rightarrow$ 9.2) to 97.8% (46.2 $\rightarrow$ 1.0) and for sTJ ABR, 6.2% (2.1 $\rightarrow$ 2.0) to 100% (40.1, 19.1, 3.9, 9.0, 6.1 $\rightarrow$ 0; 5 pts).

Conclusion: Spont. bleeds occurred a varying daily rates throughout the day-intervals following PPX dosing & did not follow any predetermined pattern.

1/week Nonacog 100 IU kg⁻¹ PPX lowered sABR and sTJ ABR vs OD in the 13 pts who experienced spont bleeding during PPX. No statistical testing was done. Over 1/3 of these 13 pts experienced no sTJ bleeding during the PPX period. Of those pts who did experience a sTJ bleed in the PPX period, 92.3% had an sTJ ABR reduction of 66.3% or more.

Disclosure of Interest: None declared.

PO258-MON

Results from the PUP-GCP clinical trial: low inhibitor rate in previously untreated patients with severe haemophilia A treated with a plasma derived FVIII concentrate

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Background: A highly purified, double virus inactivated, human plasma derived factor VIII concentrate, naturally stabilized with von Willebrand factor (pdFVIII/VWF, Octanate®), was used in this prospective clinical GCP trial. The primary objective of this study was to assess the immunogenicity of the concentrate in previously untreated patients (PUPs) and it was planned to enroll 50 PUPs with severe hemophilia A (HA) for a period of 100 exposure days (EDs), maximum 5 years.

Aims: To assess the immunogenicity of a single pdFVIII/VWF in previously untreated patients (PUPs), a prospective clinical trial has been initiated in 2000. The goal was to enroll 50 PUPs with severe hemophilia A and to follow them for an observational period of 100 exposure days.

Methods: Patients with severe HA without previous exposure to FVIII-containing products were enrolled. Inhibitors, according to modified Bethesda method, were tested prior to treatment, every 3–4 EDs (ED 1–20), and afterwards every 10 EDs (ED 21–100), but at minimum every 3 months. Efficacy and tolerability were assessed by a 4-point verbal rating scale.

Results: The study was clinically completed in spring 2014. Fifty-one subjects were enrolled and 3 of them (5.9%) developed clinically relevant inhibitor titers over the course of the study. Another 2 subjects (3.9%) displayed transient inhibitors that disappeared spontaneously without changing the FVIII dose or dosing interval. All inhibitors developed under on-demand treatment and before ED 50. The haemostatic efficacy in prophylaxis and treatment of bleeding episodes was generally rated as “excellent” and no complication was reported for any surgical treatment.

Conclusion: Despite frequent inhibitor testing and predominant on-demand treatment with the pd FVIII/VWF concentrate, the data show a low overall inhibitor rate of 9.8% in patients with severe haemophilia A, of which only three inhibitors (5.9%) were clinically relevant. No inhibitors were reported in patients mainly undergoing prophylaxis.

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PO259-MON

Designing a study for real-world data on safety and effectiveness of rixubis (Nonacog gamma) in people with hemophilia B

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Background: There is an increased need for real world data on new licensed products because of the paucity of the hemophilia B patient population involved in registration clinical trials which have rigid protocols and enrollment criteria.

Aims: To design a study that can collect data on the use of a new CHO cell line derived recombinant factor IX (nonacog gamma) in routine clinical practice reflecting real-world scenarios.

Methods: We designed a post-authorization, prospective, uncontrolled, non-interventional, multicenter cohort study aimed to describe the short- and long-term safety and effectiveness of nonacog gamma using any therapeutic regimen, in routine clinical practice (PARIXS). The primary objective is to describe hemostatic effectiveness in the prevention of bleeding events. Secondary objectives include hemostatic effectiveness of treatment of bleeding events, safety, immunogenicity, relationship between time at bleeding occurrence and estimated factor IX levels and trough levels and joint-health outcomes, impact of comorbidities and physical activity, quality of life outcomes, and use of health care resources.

Results: The study will enroll at least 80 patients with an observation period of 4 years for each enrolled subject. Recruitment is expected to start in Germany in Q2 2015.

Conclusion: This study will provide significant insight into the safety and effectiveness of nonacog gamma in routine clinical use. In addition, the study will collect long term outcomes and quality of life data for nonacog gamma.

Disclosure of Interest: None declared.

PO260-MON

Bleeding patterns evaluated during prophylaxis with a full-length pegylated recombinant factor VIII (bax 855) with extended half-life in hemophilia A

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Background: BAX 855 is a PEGylated full-length recombinant factor VIII built on ADVATE with an extended half-life. BAX 855 demonstrated efficacy and safety in prophylaxis and the treatment of bleeding episodes in previously treated patients with severe hemophilia A (Konkle et al, Haemophilia, 2015 EAHAD Congress).

Aims: Bleeding patterns during prophylaxis with BAX 855 were evaluated in the pivotal trial.

Methods: Patients' informed consent and appropriate ethics committee approvals were obtained. Patients assigned to the prophylactic arm received 45 ± 5 IU kg⁻¹ twice weekly for ≥ 50 exposure days or ~ 6 months. The per-protocol analysis included 101 subjects.

Results: At baseline, the median (range) age of patients was 29.0 (12–58) years, 68.3% of patients had 1 or more target joints (ie, a joint with ≥ 3 spontaneous bleeds in any consecutive 6-month period), and 66.3% had arthropathy. The mean (SD) reduction in dosing frequency during the study compared to pre-study prophylaxis was 26.7% (27.9). The mean (SD) annualized joint bleeding episode rate was lower in patients without target joints (1.2 [2.4]) than in those with target joints (2.2 [3.2]); however, there was no difference in rates between these groups for all bleeding events or for those occurring spontaneously. The rates between groups with and without arthropathy were similar. Approximately 60% of patients achieved ≥ 5 months between any hemorrhages, including 39.6% with zero bleeding events during treatment. For hemarthroses, approximately 80% of patients achieved ≥ 6 months between episodes, including 57.4% with zero bleeding events. The frequency of bleeding was higher at >96 h after prophylactic infusions compared with earlier times after infusions.

Conclusion: These results further demonstrate the benefit of prophylaxis and support the efficacy profile of BAX 855 for the prevention of bleeding when used twice weekly, suggesting fewer infusions may be needed for prophylactic efficacy.

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PO261-MON

Bayesian approach to the assessment of the population specific risk of inhibitors

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Background: Bayesian modelling empowers analysis of rare events, via incorporation of external data.

Aims: To illustrate how the Bayesian approach will i) compare with classical one; ii) change with different priors; and enable testing iii) thresholds and iii) size of information.

Methods: We used three different scenarios: s1) a single cohort of previously treated patients (PTPs), s2) a meta-analysis of PTPs cohorts, and s3) a previously unexplored setting (patients with positive inhibitor history).

Patient population: Hemophilia A from the Advate Post Authorization Surveillance Studies.

Outcome: Any inhibitors.

Statistical analysis: Non-informative and informative priors were applied to Bayesian standard (s1) and random-effects (s2,s3) logistic models (i,ii). Bayesian probabilities of satisfying three meaningful thresholds (10/100, 5/100 [high rates] and 1/86 [FDA mandated cut-off rate in PTPs])(iii) were estimated; and the effect of scaling up the study data size by 2 and 10 times (iv).

Results: Results based on non-informative priors were similar to the classical approach. Using priors from PTPs lowered the point estimate and narrowed the credible intervals (s1: from 1.3 [0.5, 2.7] to 0.8 [0.5, 1.1]; s2: from 1.9 [0.6, 6.0] to 0.8 [0.5, 1.1]; s3: 2.3 [0.5, 6.8] to 0.7 [0.5, 1.1]). All probabilities of satisfying a threshold of 1/86 were above 0.65. Increasing the number of patients by 2 and 10 times substantially narrowed the credible intervals for the single cohort study (1.4 [0.7, 2.3] and 1.4 [1.1, 1.8], respectively); likewise did increasing the number of studies by 2 and 10 times for the multiple-studies scenarios (s2: 1.9 [0.6, 4.0] and 1.9 [1.5, 2.6]; s3: 2.4 [0.9, 5.0] and 2.6 [1.9, 3.5], respectively).

Conclusion: The Bayesian approach provides a robust, transparent and reproducible analysis method. The risk of developing a clinically significant inhibitor in patients with a previous history of inhibitor was similar to that in PTPs.

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PO262-MON

Pharmacokinetic characteristics of factor VIII and IX concentrates – a systematic review

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Background: The efficacy of factor VIII and IX concentrates to prevent bleeds in patients with hemophilia A and B is correlated with plasma levels measured over time after the infusion. The inter-patient variability of pharmacokinetic (PK) parameters is large, but assessing individual PK profiles is hampered by the need for multiple blood samples. Population PK modeling provides a solution to this problem.

Aims: To identify PK data for factor VIII and IX concentrates to support the implementation of a population PK approach.

Methods: We conducted a literature search in MEDLINE and EMBASE from January 1997 to May 2014 for English language reports of original PK data, using the keywords "hemophilia" and "pharmacokinetics". The following data were extracted: study design, number of patients, type and severity of hemophilia, patient age, factor concentrate and dose infused, sampling data points, half-life, clearance, recovery, model used for pharmacokinetics, and inclusion of patients undergoing surgery or with inhibitors.

Results: We retrieved 753 potentially eligible articles. We included 75 articles meeting our inclusion criteria, with a total of 2050 patients included in PK analyses. 38 reported PK data on factor VIII concentrates, 25 on factor IX concentrates, and one article reported on both. Main PK parameters and their variability were summarized by class of concentrate, laboratory test technique used, sponsor of the trial, modeling characteristics as well as candidate relevant covariates. Half-life (hours) ranged from 7.8 to 19.2 for wild type, 7.5 to 17.9 for B-domain deleted and 11.5 to 23.1 for prolonged half-life factor VIII; for factor IX ranged from 12.9 to 36.0 for wild type and 53.5 to 110.4 for prolonged half-life.

Conclusion: This review provides the first systematic appraisal of the methods and results of published papers in the field. The data confirm the intra-patient variability of factor concentrate PK and support generation and adoption of population based PK models.

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PO263-MON

Immune tolerance induction with Haemate[®] p in hemophilia a: experience from a single center

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Background: Immune tolerance induction (ITI) has been shown to successfully eliminate factor VIII (FVIII) inhibitors in hemophilia A (HA) patients. A recently published review suggests that the plasma-derived, Von Willebrand factor containing FVIII concentrate Haemate[®] P may increase the probability of ITI success (Escuriola C. and Kreuz W., Haemophilia 2013; 1–7).

Aims: We evaluated data from previously untreated patients (PUPs) with severe HA who developed an inhibitor in our center.

Methods: Detailed data were collected from all PUPs who were diagnosed between 2006 and 2014.

Results: Seven out of 19 PUPs (36%) developed an inhibitor (low titer with ≤ 5 BU, $n = 4$; high titer with > 5 BU, $n = 3$). The maximal inhibitor titer ranged from 1.2 to 31 BU. The number of ED until inhibitor detection ranged from 8 to 60 (median: 27 ED). All patients (pts) were treated prophylactically with recombinant FVIII concentrates before inhibitor formation but in 4 pts an extended bleeding and in another one a surgical procedure preceded the start of prophylaxis. A high risk FVIII mutation was present in 6 pts. Whereas all low titer inhibitors disappeared during intensified prophylaxis, all 3 pts with a high titer inhibitor underwent ITI. In one patient (maximal titer 31 BU) ITI was started with a recombinant product and 5 months later switched to Haemate[®] P followed by a permanent inhibitor disappearance 2 months later. For ITI another patient with a maximal titer of 12 BU was switched initially to Haemate[®] P and was treated successfully. The third patient (maximal titer 9.8 BU) is still undergoing ITI with another plasmatic VWF-containing FVIII. Because after 7 months of ITI the inhibitor titer persists between 5 and 10 BU and considering the results from *in vitro* testing, therapy will be switched to Haemate[®] P.

Conclusion: Our data support the findings of the authors named above who conclude that Haemate[®] P is effective and produces high rates of ITI success.

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PO264-MON

2003–2014 12-year results of inherited bleeding disorders registry of the emilia romagna region in Italy

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Background: In 2003 the Health Authority of Emilia-Romagna Region (RER), developed a web-based registry to monitor epidemiology, treatment costs and improve the quality of health care of patients with Inherited Bleeding Disorders (IBD).

Aims: We provide the 12 year results of this system.

Methods: A web-based clinical record (xl'Emofilia[®]) was created to allow the 8 Haemophilia Centres (HC), organized in a 'Hub and Spoke' network, to share database, to extract detailed data and publish them on the registry (www.registroemofiliare.it) every 6 months.

Results: From 2003 to 2014 the IBD patients followed up at the HC increased from 494 to 1179 (2.3 times) while the number of RER inhabitants increased from 4 098 000 to 4 446 000 (1.08 times). The number of all diagnosis rose: Hemophilia A and B (HA, HB) from 268 to 431, VWD from 142 to 373, but mostly Rare Bleeding Disorders from 51 to 207 (10 to 18%) showing a better accuracy in the diagnosis and in the enrolment at HC. The molecular characterization of severe and moderate HA and HB patients increased from 34% to 86%, thanks to the genetic laboratory of the HUB Centre of Parma. In 2003 inhibitor patients were 11 HA, while in 2014 were 27 HA and 1HB. Prophylaxis regimen spread among patients with severe Hemophilia from 27.7% to 51.4%, while the bleeds decreased by 50%. The increase of concentrate consumption from 72 728 to 119 234 IU per patient/year was due to the spread of prophylaxis, a better recording of the home treatment, done by patients in clinical record, Immunotolerance and rise of the number of the patients.

Conclusion: Compared to 2003 there was an increase of enrolled patients (138%), inhibitor detection, genetic diagnoses and use of prophylaxis treatment. Moreover, more concentrate consumption were recorded with a considerable reduction in bleeds. In our opinion, HC network and RER Registry improved quality of care by means of detailed epidemiological reporting, quality of assistance monitoring and analyzing the quality of life and treatment costs.

Disclosure of Interest: None declared.

PO265-MON

Congenital factor deficiencies: twenty-five-year follow-up

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Background: Congenital factor deficiencies (CFD) are inherited coagulation factor deficiencies and important group of bleeding disorders.

Aims: Four hundred and seventy one CFD cases, followed and treated at our clinic between 1990 and 2015, were retrospectively evaluated in our study.

Methods: Information of patients were retrieved from patient files and from the records contained in the electronic information processing environment created after 2005.

Results: Three hundred and fifty seven of the cases were boys and 114 were girls. The ages at the admission of patients were between one week and 19 years. From the 471 cases, 129 suffered hemophilia A, 38 hemophilia B, 53 von Willebrand Disease (vWD) and 251 rare factor deficiencies (RFD). The F:C levels of patients were <1% in 122 patients, between 1 and 5% in 68 patients and between 5 and 50% in 281 patients. The problematic cases were evaluated once a month in "council of hemophilia" concerning hematologist, orthopedist, physical therapist and specialist of nuclear medicine. Ninety eight of patients were asymptomatic (47.8%) in RFD group. Asymptomatic patients were diagnosed by family histories 49(40.8%), preoperative laboratory studies 64(53.4%) and operational bleeding 7(5.8%). Seven haemophilia A patients with inhibitor had high responding inhibitor. Three hundred and twelve various surgical interventions were performed. Of the surgical interventions, 61(19.5%) were major, 57 (18.3%) were minor, 158(50.7%) were circumcision and 36(11.5%) were *radioisotope synovectomy*. Prophylaxis was applied 79 patients (57 patients with hemophilia A, 8 hemophilia B, 13 RFD and one vWD). Secondary prophylactic therapy was given to sixty eight patients with CFD. Primary prophylactic therapy was given to eleven patients with CFD. The duration of prophylaxis ranged between 4 months and 12 years (mean: 3.4 ± 4.2 year).

Conclusion: In this study, we pointed out the importance of prophylactic treatments and multidisciplinary approach in the follow-up of patients with CFD.

Disclosure of Interest: None declared.

PO266-MON

There is a direct relationship between the physical activity score and HDL cholesterol level in hemophilia patients

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Background: High-density lipoprotein(HDL) has anti-atherogenic property because it reverses cholesterol transport from the peripheral tissues to liver. Low HDL-cholesterol(HDL-C) is associated with the development of coronary heart diseases(CHD). Level of HDL-C is associated with smoking, sedentary life style, weight and nutritional status. Because of joint problems and bleeding tendency; hemophilia patients are expected to be more sedentary than normal population.

Aims: We aimed to document daily physical activity status of hemophilia patients and HDL-C relationship.

Methods: Hemophilia patients were recruited from Aegean hemophilia center. The study was approved by the Ege University ethics committees and inclusion took place after written consent had been obtained. Hemophilia A and B patients were eligible for inclusion regardless of the severity of Hemophilia. Turkish version of International physical activity questionnaire(IPAQ) was filled by the patients. The physical activity score was calculated as metabolic equivalent (MET)- min / week and classified in three levels. HDL-C levels were measured in blood samples after 8 h fasting. SPSS ver. 20 and Spearman's test was used for the statistical analysis. *P* value being <0.05 was considered statistically significant.

Results: A total of 66 hemophilia patients were included in this analysis. Mean age of the patients was 37.5 ± 11.6 . Mean activity score was 816 MET-minute/week and 44 patients had low, 14 patients had moderate and 7 patients had high activity. Mean HDL-C level was 40 mg dL⁻¹. HDL-C level and IPAQ score had positive correlations (*P* < 0.05)

Conclusion: Regular physical activity actually helps prevent bleeds and joint damage and provides strong muscles that help to lower the frequency and severity of bleeding episodes in joints and joint pain. Also aerobic exercise for 30 to 60 min on most days of the week can help to increase HDL-C. We must provide our patients safety exercise environment and good motivation that they could make it.

Disclosure of Interest: None declared.

PO267-MON

Measurement of gait variability in patients with and without hemophilic arthropathy: move-study (NTR 4561)

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Background: Hemophilia is characterized by joint and muscle bleeds that can result in cartilage destruction and irreversible arthropathy. Prophylactic replacement therapy aims to prevent arthropathy. The Hemophilia Joint Health Score (HJHS) monitors joint status. However, this is based on individual joint assessment and does not integrate the impact of multiple-joint arthropathy or muscle bleeds. Previous studies showed that video-based gait analysis can detect hemophilic arthropathy progression. Gaitsmart is a new, easily applicable, portable device that digitally measures gait.

Aims: To establish whether Gaitsmart can detect gait abnormalities in hemophilia.

Methods: Hemophilia patients ≥ 12 years without recent joint or muscle bleeds, who could walk without assistive devices were included when informed consent was given. Six sensors were mounted on calves, thighs and pelvis after which patients walked 20 meters. Gait parameters included range of motion of knee, hip, calf, thigh, pelvis; medial-lateral movement; stance flexion; joint symmetry; stride duration. A physical therapist assessed the HJHS. The study was approved by the Erasmus University Medical Center's Medical Ethics Committee.

Results: In total 106 patients participated, median age 31 years (range 12–84). The majority had hemophilia A (*n* = 93, 88%). Fifty-six patients had severe (*n* = 31) or moderate (*n* = 25) hemophilia, 30 (28%) of whom used prophylaxis. In 29 patients (27%) arthropathy was reported. Patients with clinical signs of knee or hip arthropathy (median HJHS 9, IQR 3–11) had corresponding gait deviations. Gait parameters in ankle arthropathy patients (*n* = 11) were within normal limits. Gait deviations were found more often in patients with tertiary compared to primary prophylaxis. Of 26 patients with HJHS=0, 4 had aberrant gait parameters, mainly medial-lateral movement.

Conclusion: This exploratory study suggests that Gaitsmart provides additional information to the HJHS and is able to identify hemophilia patients with joint-specific arthropathy.

Disclosure of Interest: S. Stoof: None declared, D. Hodgins Employee of: European Technology for Business, F. Leebeek: None declared, M. Cnossen: None declared, H. Horemans: None declared, S. Praet: None declared, M. Kruip Grant/Research Support from: Pfizer, unrestricted research grant.

PO268-MON

The effectiveness of exercise programme and radioisotope synovectomy: in hemophilic patientsEvim MS¹, Şekir U², Baytan B¹ and Güneş AM¹¹Pediatrics Hematology; ²Sport medicine, Uludağ University, Bursa, Turkey

Background: Repeated joint hemorrhages in hemophilic patients may lead to development of hypertrophic synovia. In some circumstances radioisotope synovectomy will become essential. Following synovectomy, an exercise programme to restore range of motion of joint is also recommended.

Aims: The aim of the study is to investigate the effectiveness of combination radioisotope synovectomy with an exercise programme on the possible improvements of the range of motion.

Methods: A total of 11 male severe hemophilic patients (mean age 14.6 ± 7.4 years) were enrolled into this study. Ten patients had Hemophilia-A and one had Hemophilia-B. Four patients had inhibitor for FVIII. All patients were receiving prophylaxis with factor concentrates before and after synovectomy. Radioisotope synovectomy with Yttrium-90 was performed in 12 knee joints. Following this procedure, the patients were advised to perform range of motion and isometric strengthening exercises 5–7 day/week for a minimum of 4 weeks at home. Range of motion exercises consisted of knee bending and knee straightening exercises. Additionally, isometric quadriceps and hamstring muscles strengthening was started. Knee joint range of motion evaluations were performed 1 week before the radioisotope synovectomy and within 2–3 months after the procedure and exercise programme with a goniometer. The deficits in knee flexion and extension were measured.

Results: We didn't observe any complication after synovectomy. There weren't new bleedings in 7 patients and the bleeding episodes in four decreased noticeably. The deficits flexion decreased significantly from $25 \pm 13^\circ$ to $14 \pm 12^\circ$ ($P = 0.009$). Similarly, the deficits extension showed a significant improvement from $7 \pm 9^\circ$ to $2 \pm 3^\circ$ ($P = 0.048$).

Conclusion: Our data confirmed the effectiveness of the combination of radioisotope synovectomy and range of motion and strengthening exercises in the treatment of chronic hemophilic synovitis.

Disclosure of Interest: None declared.

PO269-MON

Adherence to treatment in patients with haemophilia in GermanyMiesbach W¹ and Kalnins W²¹University Hospital, Frankfurt; ²DHG, Hamburg, Germany

Background: Adherence to treatment is essential in chronic diseases and haemophilia.

Aims: This study investigates adherence in members of the German haemophilia patient organisation (DHG) using the translation of the validated VERITAS-Pro questionnaire that contains six different subscales (time, dose, plan, remember, skip and communicate). Additionally, data about the social status, pain levels and co-morbidities of the patients were evaluated.

Methods: In total, 424 patients fulfilled the questionnaire (86.1% haemophilia A, 13.9% haemophilia B) of whom 89.2% had severe haemophilia. The mean age was 33.0 years (0–85 years). Mann-Whitney-U-test and Kruskal-Wallis test were used to test the association between different scores as well as the demographic and clinical data.

Results: VERITAS-Pro scores were significantly higher showing poorest adherence in patients between 20 until 59 years compared to the other age groups. Adherence to treatment was highest in patients between 0 and 14 years and between 60 and 85 years. In the patients younger than 14 years the subscales time, plan and remember showed significantly different results whereas in patients older than 60 years

subscales as time and remember differed significantly from younger patients. A multivariate analysis showed further significant differences of the score in patients with the presence of co-morbidities (hypertension), self-infusion and treatment by a haemophilia centre.

Conclusion: Adherence to treatment in patients with haemophilia differs mostly dependent on the age of the patients.

Disclosure of Interest: None declared.

PO270-MON

Prophylaxis practices in children and adults with hemophilia A or B and inhibitorsCarcao M¹, Avila M¹, Aledort L², Leissinger C³ and on behalf of Factor Utilization Expert Working Group of the International Prophylaxis Study Group IPSG and other Survey Investigators¹Pediatrics, The Hospital for Sick Children, Toronto, Canada;²The Mary Weinfeld Professor of Clinical Research in Hemophilia, Mount Sinai School of Medicine, New York; ³Tulane University School of Medicine, New Orleans, USA

Background: Prophylaxis is standard of care in persons with severe hemophilia A (HA) or B (HB) without inhibitors in many countries. In patients with inhibitors (H+I) prophylaxis is less commonly used.

Aims: We conducted a global survey to assess the extent and patterns of prophylaxis use (as of Dec 31, 2013) in this population.

Methods: Hemophilia Treatment Centers (HTCs) were asked to report on children (<18y) and adults either on, or not on immune tolerance induction (ITI), and on their use of bypassing agent prophylaxis (BA-P).

Results: One hundred and twelve centers from 14 countries in North America, Australasia and Western Europe reported on 645 H+I patients (337 children; 308 adults).

Children: 41% were on ITI alone; 17% were on both ITI and BA-P; 16% were on BA-P alone while 26% were on neither ITI nor BA-P.

Adults: 8% were on ITI alone; 4% were on both ITI and BA-P; 15% were on BA-P alone while 73% were on neither ITI nor BA-P.

Consequently 74% of H+I children were on ITI or BA-P or both while 73% of H+I adults are on neither (raising concern regarding their bleeding status). For patients not on ITI proportionally more children were on BA-P than adults; $X^2 P < 0.001$. For patients on ITI the use of BA-P was equivalent between children and adults. Overall more patients were on prophylaxis with FEIBA (149 pts) than with rFVIIa (55 pts). Among patients not on ITI an even higher proportion were on FEIBA (83 pts) vs. rFVIIa (25 pts) prophylaxis. For patients on ITI (adults and children) the most common BA-P regimen was daily for both FEIBA and rFVIIa while for patients not on ITI there was slightly more use of non-daily regimens (EOD or 3 days per week) for both rFVIIa and FEIBA.

Conclusion: This is the largest survey of patients with H+I. Use of ITI is considerably different between children and adults. Few adults are placed on ITI despite reports suggesting that ITI outcomes in adults are quite good. Similarly few adults are on BA-P. Our survey did not capture longterm patient outcome data; this needs to be evaluated.

Disclosure of Interest: None declared.

PO271-MON

Current status of older patients with hemophilia: insights from a cross-sectional pan-european study

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Background: Hemophilia centers in Europe increasingly see older patients with comorbidities as the life expectancy of people with hemophilia in developed countries improves.

Aims: The ADVANCE Working Group aimed to characterize the cohort of patients aged over 40 years with severe (<1% FVIII), moderate (1–5% FVIII) and mild (>5–40% FVIII) hemophilia by disease severity and age, in a pan-European cross-sectional study.

Methods: Clinicians in 16 centers screened a representative selection of patients in 2011/13. Following IRB approval, each center documented clinical and demographic data on consenting patients at a single clinic visit.

Results: Five hundred and nine patients with hemophilia were recruited (88% hemophilia A, 12% hemophilia B; 58% severe; 11% moderate, 31% mild). Median age was 52 years (range 35–98 years). More patients with severe than mild hemophilia were HIV positive (90/295, 30.5% vs 6/158, 3.8%), HCV positive (259/292, 89% vs 64/158, 41%), and had chronic liver disease (29.7 vs 16.6%). Irrespective of age, severe hemophilia patients had lower median BMI (25.3 vs. 26 kg m⁻²; $P < 0.0001$) and waist circumference (95 cm vs. 101 cm) than mild patients, and had lower rates of full-time employment (43.9% vs. 48.3%). Rates of malignancy were 5.3%, ischemic heart disease 5.6%, renal disease 5.4%, and diabetes 9.5%. Rates were lower in severe compared with mild hemophilia. Median creatinine clearance values (eGFR 116 ml min⁻¹ vs. 106 ml min⁻¹; $P = 0.003$) were improved and total cholesterol values (4.5 mmol l⁻¹ vs. 4.9 mmol l⁻¹; $P = 0.02$) lower in severe than mild hemophilia.

Conclusion: Historic HIV and hepatitis infection continues to affect the age distribution of the hemophilia population in Europe and the health status of affected individuals. CVD rates are low in older PWH and are more often seen in those with mild disease; they do occur in severe disease. Management advice is needed.

Disclosure of Interest: None declared.

PO272-MON

Comparing thrombin generation in patients with haemophilia A and patients on vitamin K antagonists

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Background: There is a lack of knowledge to what degree the deficiency in clotting factor VIII protects against thrombotic complications of atrial fibrillation. Therefore, it is unknown whether patients with haemophilia A (PWH) and atrial fibrillation require treatment with vitamin K antagonists (VKAs).

Aims: To investigate the haemostatic potential in PWH and patients on VKAs using thrombin generation (TG).

Methods: TG, initiated with 1 pM tissue factor, was measured with a Calibrated Automated Thrombogram in 133 patients with severe ($n = 15$) and non-severe ($n = 118$) haemophilia A, and in 81 patients on VKAs with an international normalized ratio (INR) ≥ 2.0 . All subjects were male and ≥ 18 years old. TG was expressed by lagtime, endogenous thrombin potential (ETP/area under the curve), peak, time to peak and velocity index. Results of PWH and patients on VKAs were compared according to haemophilia severity.

Results: Compared to healthy controls, all patients (PWH and patients using VKAs) had lower median ETP ($P < 0.001$). ETP in non-severe PWH was significantly higher (median 335 nM, IQR 215–519) than in patients on VKAs (156, IQR 90–225) and in those with severe haemophilia (185, IQR 116–307) ($P < 0.001$). ETP in severe PWH and patients on VKAs were comparable ($P = 0.08$). The majority (93%) of patients on VKAs had an ETP < 300 nM. Considering this threshold, 73% of severe PWH, but only 47% of non-severe PWH had an ETP < 300 nM. Other TG parameters showed similar results.

Conclusion: Although lower than in healthy controls, patients with non-severe haemophilia have a significantly better hemostatic potential than patients with severe haemophilia and patients on VKAs. Approximately half of the patients with non-severe haemophilia had higher ETP values than patients with a therapeutic INR. These results suggest that anticoagulation treatment should be considered in a large proportion of patients with non-severe haemophilia and atrial fibrillation.

Disclosure of Interest: None declared.

PO273-MON

Coated platelet levels in severe hemophilia do not correlate to bleeding phenotype

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Background: Coated platelets (CP) are a subpopulation of platelets with enhanced pro-coagulant activity following dual stimulation with agonists. The levels of CP formed in vivo are thought to play a pivotal role in hemostatic thrombus formation and structure. CP have high phosphatidylserine exposure on their surfaces and inactivated integrin GP IIb/IIIa. Normal individuals have CP in the range of 15–55%. In a single report, mean CP levels of 30% were reported in hemophilia patients; and those with a severe bleeding phenotype had significantly less compared to a mild phenotype.

Aims: To quantify CP levels in hemophilia patients versus healthy controls; and to determine whether CP level significantly differs between bleeding phenotype (mild < 5 bleeds; severe ≥ 5 bleeds per year).

Methods: Twenty subjects were enrolled including 16 severe factor VIII deficient patients (11 severe and 5 mild bleeding phenotype) and 4 healthy volunteers. The CP percentage was quantified using flow cytometry following dual stimulation with convulxin and thrombin simultaneously. Both Annexin V positive staining and PAC-1 negative staining were used to quantify CP.

Results: Mean (SD) CP levels in the control group 33(13)% were significantly higher than in hemophilia patients 17(8)%, $P = 0.004$. Both the mild and severe bleeding phenotypes had significantly lower CP levels compared to controls. The mild phenotype had mean CP of 14

(5%); the severe phenotype had 18 (8)%, which were not significantly different from one another.

Conclusion: Severe hemophilia patients have lower CP levels compared to normal, healthy volunteers. Based on the bleeding definition cutoff used in this study, CP levels did not differ among the hemophilia bleeding phenotypes.

Disclosure of Interest: None declared.

PO274-MON

Successful immune tolerance induction in an adult with hemophilia a and chronic inhibitor – a case study

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Background: Immune tolerance induction (ITI) is not often pursued in adults with hemophilia A (HA) complicated by long-standing inhibitors, as they generally have a poor likelihood of ITI success, and the inhibitor may have little effect on life expectancy. However, as age-related comorbidities and the need for general/orthopedic surgery increases, strategies to mitigate bleeding risk, including ITI therapeutic trials, become increasingly important.

Aims: The aim of this case study was to evaluate the effectiveness of ITI using a von Willebrand factor:factor VIII concentrate (VWF:FVIII) in an adult HA patient with a FVIII inhibitor.

Methods: A 21-year-old man with severe HA due to a donor splice site mutation and an inhibitor of 18 years duration presented with a history of major bleeding episodes, and a failed ITI attempt (3.5 years with recombinant FVIII). Sixteen years after the inhibitor arose, he begun a VWF:FVIII ITI regimen of 100 U kg⁻¹ daily, plus activated prothrombin complex concentrate (aPCC) prophylaxis (50 U kg⁻¹ every other day).

Results: Within 6 months, the inhibitor titer was undetectable, and FVIII activity began to rise. At this time, aPCC prophylaxis was discontinued. Within 8 months of starting VWF:FVIII ITI, the recovery of FVIII was >66% of predicted at two consecutive appointments. After 17 months of ITI, the VWF:FVIII dose was reduced by half to 50 U kg⁻¹ daily for longterm prophylaxis. Inhibitor titers have remained undetectable, and the patient has been free of bleeding symptoms.

Conclusion: ITI with a VWF:FVIII concentrate was found to be a safe, effective management approach for an adult patient with severe HA complicated by a long-standing inhibitor. A VWF:FVIII concentrate may be considered as an ITI alternative for other adults with HA and inhibitor, even after an unsuccessful attempt at ITI with recombinant FVIII.

Disclosure of Interest: None declared.

PO275-MON

Prophylactic approach to recurrent bleeding in hemophilia with inhibitors

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Background: People with haemophilia with inhibitors are at high risk for severe and uncontrolled bleeding. Prophylactic treatment with active prothrombin complex concentrate (aPCC) and recombinant factor VIIa (rFVIIa) may reduce the frequency of bleeding.

Aims: Aim is to show our prophylactic approach in haemophilia with inhibitors.

Methods: Patients with inhibitors who are on bypass-factor concentrate prophylaxis are enrolled. Age, highest inhibitor titre and bleeding frequency before and after treatment at the end of the one calendar year were recorded.

Results: Thirteen of the 24 haemophiliacs with inhibitors were treated with aPCC or rFVIIa as a prophylaxis. aPCC was given 50–75U kg⁻¹ per dose twice or three times a week and rFVIIa was given 90 µ kg⁻¹ one or twice a day. Twelve patients were on aPCC prophylaxis and one on rFVIIa. Median bleeding frequency was 4 per month (range: 2–16 per month). Nine of 12 patients on aPCC prophylaxis had a marked decrease in bleeding frequency (>75%) while one had a partial decrease (38%). One patient with an excellent response to aPCC experienced respiratory distress after aPCC infusion and prophylaxis regime was changed. aPCC did not decrease bleeding frequency in two patients and was stopped. One patient who was unresponsive to aPCC prophylaxis was given rFVIIa prophylaxis and had a partial decrease in bleeding.

Conclusion: Clinically relevant reduction in bleeding frequency was achieved with bypassing agent prophylaxis. aPCC and rFVIIa may be effective prophylactic treatment choice for patients with inhibitors.

Disclosure of Interest: None declared.

PO276-MON

Utilization of anti-inhibitors products and inhibitors status of severe hemophilia patients: 5 years national data from the Canadian hemophilia assessment and resource management system

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Background: The infusion of coagulation factor concentrates (FC) to treat patient with hemophilia, an inherited bleeding disorders, may cause the development of alloantibodies (inhibitors) against FC, reducing the effectiveness of FC. Anti-inhibitors products (anti-INH), rFVIIa and FEIBA, are infused to manage these inhibitors (INH). The Canadian Hemophilia Assessment and Resource Management System (CHARMS) tracks these infusions.

Aims: To identify the inhibitors status of severe hemophilia patients who infused anti-INH at least once over a 5 years period, the type of products infused and the reasons of infusion.

Methods: A longitudinal analysis of infusions data from hospitals and patients' bleed diaries of 25 Canadian Hemophilia Treatment Centres was conducted using the national CHARMS database annual subsets.

Results: From 2009 to 2013, 83 severe male hemophilia patients (94% Hemophilia A) infused anti-INH at least once. At their first infusion during the study period, 42% were adult. A total of 37 patients (45%) had 5 years of data available. During these 5 years, 15 patients were always recorded as INH negative(-) [18%, group 1], 51 remained INH positive(+) [61%, group 2], 5 became INH+ [6%, group 3] and 12 went from INH+ to INH- [14%, group 4]. Overall, 67% of patient infused anti-INH and FC, 33% infused anti-INH exclusively. The proportions of patients who infused both rFVIIa and FEIBA was 40% in group 1, 57% in group 2, 33% in group 3 and 20% in group 4; there was no significant difference between these proportions ($P = 0.08$). The proportion of patients who infused anti-INH only for prophylaxis was 27% in group 1, 26% in group 2, 33% in group 3 and 0% in group 4.

Conclusion: The majority of patients who infused anti-INH were recorded as inhibitors positive at least once. Most patients did not infused solely one brand of anti-INH, they infused both brands or with FC. Only a quarter of the patients infused anti-INH products exclusively for prophylaxis.

Disclosure of Interest: None declared.

PO277-MON

Reliability of patient-reported outcome (PRO) instruments in US adult people with hemophilia (pwh): the pain functional impairment and quality of life (P-FIQ) study

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Background: Hemophilia is marked by frequent hemarthrosis, resulting in pain and functional impairment. Standardized and disease-specific patient-reported outcome (PRO) instruments have been used in studies of PWH but not validated.

Aims: To assess reliability of PRO instruments in nonbleeding PWH.

Methods: Sequential adult male PWH (mild-severe) with a history of joint pain or bleeding completed a pain history and PROs (EQ-5D-5L, Brief Pain Inventory [BPI], SF-36, International Physical Activity Questionnaire [IPAQ], and Hemophilia Activities List [HAL]) during routine visits. Patients were initially asked to complete the PROs again at the end of a ~3–4 h visit until ~150 had done so.

Results: Three hundred and eighty one patients enrolled between 10/2013 and 10/2014; 164 (87.7% of initial 187) completed the retest. Median age of the retest cohort was 33.9 (Q1, Q3, 26.9, 46.0). Median time for completion of initial survey/PROs was 36.0 min and for PRO retest was 21.0 min. Most had hemophilia A (74.4%), were white non-Hispanic (72.6%), and employed (80.7%); 48.7% were married, and 61.0% were overweight or obese. More reported HCV (49.4%) than HIV (16.5%); 61.0% self-reported arthritis/bone/joint problems. Median test-retest concordance of individual items was EQ-5D-5L, 80.0%; BPI, 54.5%; SF-36, 77.8%; IPAQ, 100.0%, and HAL, 77.4%. Median time between tests was 1.52 h. Intraclass correlation coefficients for test-retest reliability were EQ-5D-5L Index, 0.890; BPI – severity, 0.950; BPI – interference, 0.920; SF-36 overall health, 0.910 (subscores 0.760–0.960); IPAQ total activity, 0.940 (vigorous 0.950, moderate 0.980); HAL total score, 0.970 (subscores 0.780–0.960).

Conclusion: All 5 PRO instruments appear to be reliable and provide different levels of detail in describing impact of hemophilia on pain and function and consequently have varied burden of administration. Specific instruments should therefore be tailored to the study design or clinical need for specific outcome assessment.

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PO278-MON

Acquired hemophilia in patient with chronic myelomonocytic leukemia (CMML1)-a case report

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Background: Acquired hemophilia (AH) is one of the most severe diathesis haemorrhagica. It is caused by IgG autoantibodies interfering against the A2 or C2 domain of factor VIII and decreasing its coagulant activity. AH is characterized by severe bleeding diathesis (gastrointestinal or genitourinary hemorrhage). Pathogenesis of inhibitor is unknown but it can be associated with other autoimmune disorders and the older age suggest immune dysregulation. AH can coexist with malignances, infections, medications or pregnancies.

Aims: Acquired hemophilia in patient with chronic myelomonocytic leukemia (CMML1).

Methods: Patient 63y suffering from lichen planus, nephrolithiasis, steroid osteoporosis complicated left side costa VII and vertebra Th7 fracture and absolute vertebral canal stenosis, after acute pancreatitis and implant of aortal-femoral prosthesis was admitted in a good condition for severe hemorrhagic diathesis of upper and lower limbs, splenomegaly. Results of laboratory tests: factor VIII activity-2%, factor VIII antibodies -8.5 Bethesda Units mL⁻¹, correction test APTT was positive, WBC-77.33 G L⁻¹ (blood smear: myelocytes-7%, metamyelocytes-20%, Bands-6%, Segmented-44%, lymphocytes-11%, monocytes-12%), anemia-II° WHO (Hb-9.9 g dL⁻¹), FAG-0, OB-70. No JAK2 V617V and BCR-ABL p190, p210, p230 t(9;22)(q34;q11) mutations were detected. Karyotype was normal, bone marrow biopsy showed CMML1 manifestation.

Results: Due to active bleeding diathesis treatment with activated prothrombin complex concentrates at doses of 50 units every 12 h for 3 days, than Prednisone in dose 1 mg kg⁻¹ for 6 weeks were performed. Clinical improvement was achieved (no bleeding diathesis, increase of factor VIII activity to 43%). After Prednisone Hydroxyurea (1.0 g daily) was administered. Overall response was 13 months and overall survival was 17 months.

Conclusion: Treatment of acquired hemophilia with aPCC and steroids allowed to achieved clinical response and Hydrea therapy for CMML1 could be introduced.

Disclosure of Interest: None declared.

PO279-MON

Results of radiosynovectomy in children with severe hemophilia and factor VIII or IX inhibitor

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Background: One of the complications of bleeds in children with severe hemophilia and factor VIII or IX inhibitor is arthropathy.

Aims: To evaluate the clinical benefits of radiosynovectomy (RS) in children with hemophilia, reduce bleeds and improve progression free survival (PFS).

Methods: Seven patients aged 9–16 years, (mean age 13.6 years) were included in the study 5 had hemophilia A with inhibitor factor VIII and two had hemophilia B with inhibitor factor IX. 186 Reccoloid was injected. Into 6 knee joints and 5 elbow joints In three cases injections

were repeated once due to recurrence bleeds. Activity of injected ^{186}Re colloid was dependent on type and size of joints (range: 60–180 MBq). All patients received Novoseven in doses of 90/100 lg kg⁻¹ before the injection. The next doses were given after 2 h, after 3 h and then again after 3 h. FEIBA was administered 3 h later. No bleeding into joints was observed for 2 days after surgery. Post therapy imaging was performed in each case 1 h after injection of radioisotopes, and then on day 2 and 3. The number of bleedings into joints per month, improvement of joint movements, and other clinical changes were evaluated before and after therapy in 3 months intervals. US images were used to assess changes in joint effusion and synovial overgrowth. PFS in terms of significant clinical improvement within joints was assessed using standard Kaplan Meier methods. The prognostic significance of selected parameters was tested using discriminate function analysis.

Results: After injection of ^{186}Re into joints, post-therapeutic images showed intraarticular distribution of tracer and there was no leakage. A significant reduction in bleeds into joints was noted before therapy (mean 1.7 per month) and after treatment (mean 0.6 per month, $P < 0.001$).

Conclusion: This study suggests that the use of ^{186}Re in early stages of arthropathic haemophilia reduces the number of bleeds into joints and improves PFS.

Disclosure of Interest: None declared.

PO280-MON

F8 mutation and inhibitor development in severe factor VIII deficiency: results from the Irish national database

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Background: Neutralising inhibitory antibodies to factor VIII therapy is the most significant challenge in the treatment of severe haemophilia A. Inhibitor development is influenced by heritable and treatment related issues with the *F8* mutation one of the most significant predisposing factors.

Aims: The Irish haemophilia A database was reviewed to determine the association between inhibitor occurrence and *F8* gene mutation.

Methods: *F8* mutations and the presence or absence of FVIII inhibitor and levels were determined by standard methods.

Results: Data was available on 177 of 185 patients registered with severe haemophilia A. *F8* mutations were identified in 176 patients; comprising 65 *F8* Int22 h inversion events (37%); 47 small indels (27%), 47 missense mutations (27%), 8 nonsense mutations (4.5%), 4 patients with deletions of one exon or more (2%) and 2 patients with splice site mutations (1%). 45 patients were described as inhibitor positive. 20 of 45 are high responders (>5 BU), as measured by the Bethesda assay, the remaining 25 are low responders (<5BU). Mutation data was available on all patients with inhibitors; 2 of 4 patients with gross deletions (50%), and 24 of 67 *F8*Int22 h inversion patients were inhibitor positive (36%). These two mutation types were present in the inhibitor positive cohort at a higher frequency than in the inhibitor negative group. 9 of 49 patients with small indels (18%); 1/8 with nonsense mutations (12.5%), 9/47 patients (19%) with missense mutations, 0/2 patients with splice site mutations were inhibitor positive. These patient groups were present at a lower frequency in the inhibitor positive group compared to the inhibitor negative group. Six different mutations were identified in the 9 patients with missense mutations and inhibitors. There was no apparent difference between mutation types in the low and high responders.

Conclusion: Gross deletions and the *F8* intron 22 h inversion events confer the highest risk of inhibitor development to patients with severe haemophilia A in Ireland.

Disclosure of Interest: None declared.

PO281-MON

Patient preferences for FVIII and BAX 855: results from the BAX 855 pivotal trial

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Background: BAX 855 is pegylated recombinant Factor VIII based on ADVATE. It was developed as an option to further personalize care and improve outcomes in people with hemophilia A. As a new therapy, it is important to understand patient satisfaction and preferences relative to their prior treatment.

Aims: To describe patient satisfaction with and preference for FVIII and BAX 855 as reported in the pivotal trial.

Methods: BAX 855 was studied in a 6-month, phase 2/3, multi-center, open label study. Subjects in Arm A received 45 ± 5 IU/kg of BAX 855 twice weekly while subjects in Arm B infused BAX 855 on-demand. Patient preference and satisfaction were included as exploratory endpoints. Patient satisfaction was captured using a 5-item Likert scale at baseline for their prior FVIII therapy and at follow-up for BAX 855. Patient preference for BAX 855 or their prior FVIII therapy was captured at follow-up. IRB approval and informed consent were obtained. The percent of very satisfied and satisfied patients were combined for this analysis. Results were reported for all patients and by treatment arm.

Results: One hundred and thirty eight patients were in the intent to treat (ITT) sample. The majority of patients in the ITT sample completed the satisfaction questionnaire, however the number varied slightly by question and visit. While 79.4% (100 of 126) of patients were satisfied with their prior FVIII at baseline, satisfaction increased to 90.1% (109 of 121) with BAX 855 and 84.7% (100 of 118) preferred BAX 855 over their prior FVIII. Of the 85 patients in Arm A previously on prophylaxis, 18 patients in Arm A previously on-demand, and 15 patients in Arm B previously on-demand, 83.5%, 88.9% and 86.7% preferred BAX 855 over their prior FVIII, respectively.

Conclusion: Notably, patients enrolled in the BAX 855 trial had a high degree of satisfaction with their prior FVIII therapy. Nonetheless, treatment with BAX 855 was successful in further improving patient satisfaction with the majority of patients preferring it over their prior FVIII product.

Disclosure of Interest: J. Epstein Shareholder of: Baxter, Employee of: Baxter, E. Schwartz Shareholder of: Baxter, Employee of: Baxter, B. Abbuehl Shareholder of: Baxter, Employee of: Baxter, B. Ewenstein Shareholder of: Baxter, Employee of: Baxter.

PO282-MON

Recurrent hematuria in hemophilia patients is not indicative of the presence of an underlying cause

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Background: The incidence of hematuria in the general population in the Netherlands is 2.6%. In our hemophilia treatment center several hemophilia patients present with hematuria each year. In order to receive adequate treatment and identify a possible underlying urological or renal problem, a multi-disciplinary approach is needed. The WFH guideline recommends clotting factor concentrate administra-

tion when hematuria persists and evaluation by a urologist in case of recurrent episodes.

Aims: To study the prevalence and treatment of hematuria in hemophilia A and B patients in our center with the overall aim to evaluate the WFH guideline.

Methods: Records of all hemophilia A and B patients were evaluated between 1983–2014 for hematuria episodes and corresponding treatment regimens.

Results: Of 245 hemophilia patients, 36 (15%) developed hematuria of which the first episode was at a median age of 36 years (range 11–87). Eighteen patients (50%) received clotting factor concentrates, 10 of which had recurrent hematuria. Twenty-eight patients (78%) were referred to a urologist and received additional examinations (urine analysis, bladder and upper urinary tract imaging, CT-scan, cystoscopy). Of the 16 patients with first episode of hematuria, 12 were referred (75%) and 16 of 20 patients with recurrent hematuria were referred (80%). Thirteen of 28 patients (46%) had an underlying cause of hematuria, such as kidney stones, cystitis or angiomyolipoma. An underlying cause was found as frequently in patients with recurrent hematuria compared to a single episode. Patients in whom an underlying cause was identified were similar compared to patients without an identified cause of hematuria.

Conclusion: These preliminary results show underlying problems in around 50% of hemophilia patients presenting with hematuria, regardless of a first or recurrent episode. We suggest additional investigations in case of hematuria irrespective of age or number of bleeding episodes to identify and treat underlying disorders.

Disclosure of Interest: None declared.

PO283-MON

Spontaneous intracranial bleeding is a major cause of death in nonsevere hemophilia A patients

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Background: Life expectancy of nonsevere hemophilia A patients without hepatitis or HIV equals the life expectancy of the non-hemophilic population. However, data on hemophilia-related causes of death in nonsevere hemophilia A patients are scarce. They may provide insight in areas of care that can be improved.

Aims: To analyze bleeding-related causes of death in nonsevere hemophilia A patients.

Methods: The INSIGHT study is an international cohort study of all nonsevere hemophilia A patients that were treated with factor VIII concentrates during the observation period between 1980 and 2010 in 34 participating centers across Europe and Australia. Clinical data and vital status were collected from 2709 patients. The 10th revision of the International Classification of Diseases, Injuries, and Causes of

Death of the World Health Organization is used to classify the primary and secondary cause of death.

Results: In 62 of the 148 patients (42%) that died during the observation period, the cause of death was related to hemophilia A. In this group of 62 patients, 28 (45%) patients died from complications related to hepatitis B/C (29%) or HIV (16%). In 34 patients (55%), death was caused by bleeding which was an intracranial hemorrhage in 20 (32%). Intracranial hemorrhage occurred spontaneously in 16 (26%).

Conclusion: In this cohort the cause of death is hemophilia-related in nearly half (42%) of the deceased patients; spontaneous intracranial bleeding being a major cause of death. These results stress the severity of nonsevere hemophilia and the importance of early presentation to specialized care. With the surprisingly high incidence of spontaneous intracranial hemorrhage that was observed in this group, other risk factors for intracranial hemorrhage may also have been present.

Disclosure of Interest: None declared.

PO284-MON

Reliable RNA retrieval from low-volume paxgene tubes in simulated "real world" next generation sequencing (RNA-SEQ): Gena-05 substudy pilot

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Background: Evaluation of the dynamic changes in gene expression (RNA-Seq) at first exposure to Factor VIII in previously untreated patients with severe haemophilia A may provide early markers of inhibitor risk and mechanistic insights. Due to RNA fragility, there is a requirement for stabilisation at the point of sampling. We evaluated a modified low volume sample tube (1 mL) to assess feasibility of paediatric use.

Aims: To assess RNA (mRNA & miRNA) quantity (concentration) and quality (integrity: RIN) obtained using a modified PAXgene RNA tube and the effect of simulated pre-analytical variables.

Methods: Whole blood samples from healthy adult controls ($n = 20$) were obtained with informed consent using the 1 mL modified (M) or 2.5 mL standard (S) RNA tube. The effect of sample volume, incubation and freeze time were also evaluated.

Results: Comparison of modified and standard tubes demonstrated a significant difference in the RNA concentration obtained (M 66.5 v S 153.0 ng μL^{-1} , $P = 0.0002$) and a small, but significant difference in RIN (M 6.8 vs. S 7.8, $P = 0.02$). RNA concentration was significantly affected by incubation time and sample volume, but not freezing. RNA integrity was not significantly affected by incubation time, freezing or sample volume. No correlation was seen between reporter gene expression (DNA topoisomerase 1) and RNA concentration or integrity. Subsequent study phases to optimise RNA extraction demonstrated higher mean integrities (5.9 v 7.4 v 8.4, $P < 0.0001$) with some variability in RNA concentration (74.4 v 137.5 v 58.0 ng μL^{-1} , $P = 0.005$). Recruitment to date in the GENA-05 RNA-Seq substudy using this modified tube has accrued 95 samples from 27 patients from sites in 6 countries.

Conclusion: Although lower concentrations of RNA were obtained from assaying a lower blood volume, both the quantity and integrity was maintained above that required for downstream analysis by RNA-Seq. This modified tube offers a simple, low volume solution for mRNA/miRNA sampling in paediatric studies.

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PO285-MON

U.S. surveillance of prophylaxis use among persons with hemophilia A receiving care at hemophilia treatment centers (HTCS)

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Background: Prophylactic replacement of clotting factor has been demonstrated as effective in the prevention of bleeding and arthropathy in patients with severe hemophilia A (sHA). The World Federation of Hemophilia and the National Hemophilia Foundation recommend that primary prophylaxis be considered as first-line therapy for patients with sHA and is defined as regular continuous treatment initiated in the absence of documented joint disease started before the second clinically evident joint bleed and age 3 years.

Aims: To determine the use of continuous prophylaxis (CP) and the proportion of patients who began CP prior to the age of 3 among sHA patients participating in the Community Counts (CC) project in the USA Hemophilia Treatment Center Network (USHTCN).

Methods: From December 2013-January 2015, sHA patients at 81 HTCs were enrolled in the CC Registry, an initiative of the Centers for Disease Control and Prevention, the American Thrombosis and Hemostasis Network and the USHTCN to monitor complications of bleeding disorders. Data was collected about the patients' treatment regimen and, if on CP, the age at which CP was first started.

Results: Of the 867 sHA patients enrolled, 72.1% were on a CP regimen; episodic/on-demand (20.8%); event-based, short-term or intermittent prophylaxis (3.5%); Immune Tolerance Induction (ITI) (2.1%); and CP with by-passing agents plus ITI (1.6%). 237(37.9%) sHA patients on CP began prophylaxis before the age of 3, of whom 9% were less than 3, 34% were age 3–9 and 41% were age 10–19. More than half of adults over age 20 and without an inhibitor used CP.

Conclusion: National practice trends and outcomes in hemophilia can be determined using the CC Registry. The CC project enhances our ability to measure whether treatment recommendations are translated into clinical practice for patients receiving care through the USHTCN. Bleeding events and barriers to the 24% of sHA patients not using CP require further exploration.

Disclosure of Interest: None declared.

PO286-MON

Assessment of inhibitor risk after switching from plasma-derived factor VIII concentrate to recombinant factor VIII (Brasil-RFVIII: brazilian study of inhibitor linked to recombinant factor VIII)

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Background: Inhibitors are one of the major complications of hemophilia A (HA) treatment. The impact of product switching on factor VIII immunogenicity remains controversial. In Brazil, recombinant factor VIII concentrate (rFVIII) became available since August 2013 for all HA patients under 30y.

Aims: To evaluate the occurrence of inhibitors in HA patients after switching from plasma-derived FVIII concentrate (pdFVIII) to rFVIII.

Methods: Previously treated patients (PTP) with HA (FVIII <2UI dL⁻¹) were enrolled in this study when switched from pdFVIII to rFVIII (Advate® Baxter). Blood samples were collected to evaluate the presence of inhibitor, immediately before switching and after 10, 20 and 50 exposure days (ED) to rFVIII. Patients with detectable inhibitor (>0.6BU) immediately before switching were excluded. Inhibitor quantification >0.6BU in two consecutive samples was considered positive.

Results: Three hundred and forty six HA patients, mean age 12.6 ± 14 (11mo to 31y) completed 50DE to rFVIII. Overall 5 (1.5%) patients had inhibitor presence confirmed in more than one sample. Two cases, 4 and 5y, with >100ED to pdFVIII before switching showed low titer inhibitor with good response to prophylactic treatment with high dose rFVIII. Two patients (9mo and 11mo) with history of 2 and 7ED to pdFVIII before switching presented high-titer inhibitors (240BU and 15BU, respectively). The last patient was 5y, had 30ED to pdFVIII before switching, and developed high titer inhibitor (7.2BU) after 15ED to rFVIII.

Conclusion: In Brazil, switching among several different pdFVIII products is common. Recently, rFVIII became available in Brazil, representing a valuable opportunity for assessment of inhibitor risk related to product switching (pdFVIII to rFVIII). In this study, all patients with high-titer inhibitor were under 5y, and had less than 30ED to pdFVIII before switching. Thus, it is not possible to determine the role of product switching in clinically significant inhibitor development.

Disclosure of Interest: None declared.

PO287-MON

Phenotypic profile of a child with severe hemophilia B and inhibitors with novel mutation in the FIX (F9) gene

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Background: HEMOPHILLIA B(HB) is X-linked recessive bleeding disorder, caused by mutations in the factor IX (F9) gene. It occurs in

about 1:30 000 male births. Inhibitors occur in 1.5–3% of all persons with HB, majority of persons who develop inhibitors have severe HB. Our department is part of HTC Skopje where all children with bleeding disorders in our country are diagnosed and controlled. There are 56 children (0–18 years) with hemophilia, 22 with HB, 2 severe HB and one HB with inhibitors.

Aims: Presentation of clinical manifestations of the child with severe HB and inhibitors, who has novel mutation in the FIX(F9) gene.

Methods: Clinical assessment and echosonography was used for muscle bleedings; FIX activity was determined with one stage clotting assay; Inhibitors were determined by Bethesda coagulation method. Direct sequencing of the FIX gene coding regions using BigDye v1.1 Termination kit was performed for determination of the molecular defect.

Results: The child with severe HB, FIX activity <0.2%, was diagnosed at 2.5 years of age. He has novel mutation c.230_231delTT insA, p.Val77Aspfs*27, Exon b (2). The first episode of bleeding was treated with pdFIX and continued with prophylaxis 30 IU kg⁻¹ BW, 2 x weekly. After 17 ED minimal rise of inhibitors was registered (0.15 BU). After 21 ED and 24 ED the inhibitors raised on 1.9 BU and 4.0 BU. Next bleedings were treated with bolus doses of rFVIIa 270 µg kg⁻¹ (followed by decreasing of inhibitors) or with pdFIX (only for two haemarthroses, followed by rising of inhibitors). It was not registered allergic reaction. During 5 years he had 1 major and 10 minor muscle bleedings and 11 haemarthroses of the ankles and toes.

Conclusion: The child with severe HB and novel FIX(9) gene mutation, c.230_231delTT insA, p.Val77Aspfs*27, Exon b (2), had no allergic reactions on pdFIX and developed inhibitors early. He showed rare, mainly muscle bleedings and the treatment was successful with bolus doses of rFVIIa 270 µg kg⁻¹.

Disclosure of Interest: None declared.

PO288-MON

Mild hemophilia a complicated by inhibitor after surgery and factor replacement therapy

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Background: The clinical landscape of the management of patients with mild Hemophilia A has been altered due to the development of inhibitors which present later in life (peak incidence at 66 years) than in those with severe disease. Their clinical presentation can vary from mild to life-threatening and its treatment can be quite challenging.

Aims: Sixty y/o gentleman with PMH of Hemophilia A FVIII: 7% had a recent hemi-colectomy for a tubular adenoma with a prolonged hospital stay complicated by acute respiratory failure, acute renal failure, septic shock, ischemic bowel and retroperitoneal bleed requiring multiple revision surgeries and an extensive rehabilitation. He was discharged home with FVIII prophylactic infusions.

Methods: Prior to this episode, he had rarely required FVIII and had no history of spontaneous bleeds and his inhibitor assay was negative. Two weeks after discharge, he presented to the ED with abdominal pain accompanied severe anemia and a hemoglobin of 4 gm dL⁻¹ and was diagnosed with a massive retroperitoneal bleed with areas of active arterial extravasation beginning from the right mid-abdomen adjacent to the surgical clips from his prior surgery, extending to the right pelvis. His factor activity was noted to be <1%. A bypassing agent was started immediate with the suspicion of an inhibitor and guided coil embolization of the branches of the pancreaticoduodenal, middle colic and right colic arteries. His inhibitor titers were initially 3.9 BU on admission, by D10 had increase to 7.3 BU.

Results: Concomitantly with bypassing agents, immune tolerance with FVIII was started and by day 30 his inhibitor was negative. He remained with FVIII infusions without any further bleeding.

Conclusion: The development of inhibitors in patients with mild hemophilia A can complicate their clinical presentation and makes their management challenging. It is important that we identify the risk factors for inhibitor development, have alternative agents available and a multi-disciplinary care team.

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Heparin and heparinoids

PO290-MON

Disturbance of the hemostatic balance by cardiopulmonary bypass surgery

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Background: The hemostatic system is disturbed in patients undergoing cardiac surgery with cardiopulmonary bypass (CPB). Hemodilution occurs due to the CPB procedure, coagulation factors are consumed, and high doses of unfractionated heparin cause an elevated antithrombin (AT) consumption. Because both pro- and anticoagulant mechanisms are affected during surgery, it is difficult to determine their individual effect on thrombin generation (TG).

Aims: Using a new approach to analyze the underlying processes of TG separately, we aimed to determine the effect of CPB procedure on prothrombin conversion and thrombin inactivation.

Methods: Plasma was collected from 30 patients undergoing CPB before and after surgery. AT, α₂Macroglobulin (α₂M), and prothrombin levels were determined. TG was measured and prothrombin conversion and thrombin inactivation curves were extracted using a validated algorithm.

Results: TG is reduced post-surgery as compared to pre-surgery (peak 226 ± 64 vs. 320 ± 62 nM; *P* < 0.001), and the plasma levels of AT, α₂M and prothrombin are decreased (*P* < 0.001). The total amount of prothrombin converted during TG is reduced after surgery (402 ± 218 vs. 701 ± 181 nM; *P* < 0.001) and the maximum prothrombin conversion rate is reduced post-surgery as well (315 ± 69 vs. 180 ± 49 nM/min). The thrombin decay capacity was lower after surgery (0.68 ± 0.16 vs. 0.43 ± 0.23 min⁻¹), resulting in less thrombin-AT (53%; *P* < 0.001) and less thrombin-α₂M formation (85%; *P* < 0.001). The *in silico* normalization of prothrombin conversion increased the TG peak height above pre-surgical levels (143%). However, a slight increase of prothrombin conversion (up to 83% of the pre-surgery level) restores TG peak height to the pre-surgery level.

Conclusion: Pro- and anticoagulant mechanisms are attenuated during CPB surgery. The hemostatic balance shifts toward bleeding, as prothrombin conversion and TG are reduced after surgery. TG can be restored to pre-surgical levels by slightly increasing prothrombin conversion.

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PO291-MON

Bovine and porcine mucosal heparins exhibit similar biologic profiles

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Background: In consideration of the limited supply of porcine heparin (PH), which may result in a global shortage, the current discussions on the reintroduction of bovine heparin (BH) are timely and justified.

Aims: This study compared five different batches each of BHs and PHs both of mucosal origin.

Methods: Commercially available PHs and BHs were obtained from various US and Brazilian vendors. USP reference standard heparin (Lot F01187) was used to compare the potency of these two groups of heparin utilizing ACT, aPTT, Heptest, Calcium Thrombin Time, anti-Xa and anti-IIa activities, as well as protamine and PF4 neutralization. The interaction with HIT antibodies was studied using a pooled HIT sera preparation. The molecular weight profile was determined by HPLC.

Results: On a gravimetric basis, the anticoagulant activity of PH was 10–30% higher in comparison to BH. PH exhibited higher anti-Xa and anti-IIa activities. The USP cross referenced potency in the anti-Xa assay was $160 \pm 20 \text{ U mg}^{-1}$ for PH vs. $132 \pm 16 \text{ U mg}^{-1}$ for BH. Protamine and PF4 titration profiles were comparable. In the HIT assay, there was no difference in between the two groups. The molecular weight of BH was $17.1 \pm 0.8 \text{ kDa}$, whereas that of PH was $16.6 \pm 0.5 \text{ kDa}$ ($P > 0.05$).

Conclusion: While PH exhibits relatively stronger anticoagulant and anti-protease activities, in other assays both BH and PH exhibit comparable compositional and functional profiles. Thus, BH may be considered as biosimilar to PH and can be accordingly defined and developed for clinical use.

Disclosure of Interest: None declared.

PO292-MON

Characterization of the antithrombotic fingerprint of the branded and copies of the low molecular weight enoxaparin using thrombin generation assay

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Background: The patent protection of LMWHs expired so the definition of criteria for the biological similarity between LMWH copies and the original product is a real need.

Aims: The present *in vitro* study, compared copies and branded enoxaparin using the specific anti-Xa activity and the calibrated automated thrombogram assay.

Methods: Samples of platelet poor (PPP) and platelet rich plasma (PRP) from 15 healthy volunteers were spiked with branded enoxaparin (Lovenox®) or its copies (Cutenox®, DilutoI®, Enox®, Fibrinox®, Loparin®, Lupenox®, Novex®, Noxprin®, Versa®). The specific anti-Xa activity was measured in PPP and thrombin generation was

assessed in PPP and PRP in the presence of tissue factor or pancreatic cancer cells BXP3.

Results: The anti-Xa activity of enoxaparin copies ranged from 0.072 to 0.088 IU μg^{-1} , being lower as compared to the branded enoxaparin (0.095 anti-Xa IU μg^{-1}). The potency of each copy to inhibit thrombin generation varied in the three experimental systems. The presence of platelets or pancreatic cancer cells BXP3 in human plasma induced significant modifications of the inhibitory efficiency of enoxaparin copies on thrombin generation which distinguished them from the branded product.

Conclusion: Enoxaparin copies showed significant variability regarding their inhibitory potency on thrombin generation. Platelets and cancer cells significantly increased the variability of the antithrombotic efficiency of the copies as compared to the branded enoxaparin. The present study underlines the need for the elaboration of additional functional criteria to evaluate the global antithrombotic capacity of enoxaparin copies in order to evaluate their potential sameness with the branded drug.

Disclosure of Interest: None declared.

PO293-MON

Comparative studies on the molecular and functional profile of sheep mucosal derived enoxaparin with varying Anhydro-Manno Groups

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Background: Enoxaparin is the most commonly used Low Molecular weight heparin which is derived from Porcine mucosal tissues. More recently Enoxaparin preparations derived from Bovine mucosal Heparin and Sheep Mucosal heparin have become available.

Aims: The purpose of this study was to compare 3 sheep enoxaparin preparations with varying amounts of anhydromanno contents with branded enoxaparin in routinely carried out biological assays.

Methods: Enoxaparins of sheep origin were obtained from Ronssi Pharmaceuticals China. Branded enoxaparin was purchased commercially from Sanofi Aventis. Normal human plasma was supplemented with each of these agents individually to obtain final concentrations of 0–10 $\mu\text{g mL}^{-1}$ to profile the anticoagulant and anti-protease activities. Protamine neutralization studies were carried out. HIT antibody mediated platelet aggregation studies were also carried out

Results: The molecular profile of the individual sheep enoxaparins irrespective of the anhydromanno component was comparable to branded enoxaparins (4.1–4.3kDa vs. 4.2kDa). In the global tests such as the aPTT, Heptest and Thrombin time both the branded and sheep heparin provide comparable results. Similarly in the protamine neutralization studies comparable neutralization patterns were noted in different tests. In the Anti-xa assay the relative potency for the sheep enoxaparin ($98\text{--}104 \text{ U mg}^{-1}$) compared to branded enoxaparin (99 U mg^{-1}). In the HIT studies all agents exhibited comparable responses. The anhydromanno content did not produce any significant differences in the functional profiles of sheep enoxaparins.

Conclusion: These results indicate that the sheep mucosal heparin derived enoxaparin exhibit comparable molecular profile and biological activities in the laboratory assays. Based on these studies it is concluded the sheep mucosal heparin is comparable to porcine mucosal heparin derived enoxaparin. Furthermore, varying content of anhydromanno group did not alter the functional profiles of sheep heparins.

Disclosure of Interest: None declared.

PO294-MON

Sevuparin; effects on hemostasis of a novel polysaccharide drug derived from heparin

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Background: Unfractionated heparin (UFH) has previously been found beneficial in the treatment of sickle cell disease (SCD) and malaria, but the complication of bleeding limits its use. In sevuparin, being developed for normalising blood flow during vasoocclusion, the inherent antiadhesive properties of UFH are preserved while the pentasaccharide required for antithrombin (AT) binding is abolished. The next step in sevuparin development is the start of a phase II clinical trial in SCD.

Aims: To investigate the residual effects of sevuparin on hemostasis.

Methods: Anticoagulant profiling by plasma- and purified reagent-based assays; bleeding side effect assessment in a rat bleeding model and hemostatic effects in a phase I clinical trial were studied with sevuparin.

Results: *In vitro*, the specific activity of sevuparin was 4 IU mg⁻¹ by AT dependent anti-Xa and anti-IIa assays (cf. UFH 200 IU mg⁻¹) and by APTT 15 IU mg⁻¹. Further APTT analysis confirmed that effect was elicited mainly via Heparin Cofactor II (HCII), with contribution through AT. Sevuparin did not directly affect thrombin or anti-Xa activity and had no effect on fibrinogen conversion. Studies with platelets did not show any effect neither on activation nor aggregation. *In vivo*, in the rat bleeding time model ED50 for sevuparin was more than 75-fold higher than for UFH and 10-fold higher than for LMWH. For the APTT response the corresponding dose by weight is more than 35-fold higher than for UFH and 5-fold higher for LMWH. In the phase I study, intravenous doses up to 360 mg 4 times daily were given to 25 male healthy volunteers; sevuparin induced a transient linear, dose-proportional increase in APTT up to 2-fold the normal value while the values at 2 h after injection were not significantly elevated in any subject.

Conclusion: The low residual effect of sevuparin on hemostasis is explained by mechanisms involving HCII and possibly by a pentasaccharide-independent potentiation of AT, causing an transient increase in APTT which can be monitored in the clinic.

Disclosure of Interest: M. Lindgren Employee of: Dilaforette AB, J. Meijers Grant/Research Support from: Dilaforette AB, B. Biemond Consultant for: Dilaforette AB, S. Ramström Grant/Research Support from: Dilaforette AB, T. Lindahl Grant/Research Support from: Dilaforette AB, P.-O. Eriksson Shareholder of: Dilaforette AB, A. Leitgeb Employee of: Dilaforette AB, M. Wahlgren Shareholder of: Dilaforette AB, J. Hogwood: None declared, E. Gray: None declared, E. Holmer Shareholder of: Dilaforette AB

PO295-MON

Reversal of the heparinoid effect with prothrombin complex concentrate – an in vitro study using the thrombin generation assay

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Background: Heparinoids are used both as a prophylactic measure and in the acute treatment of VTE. Reversal of anticoagulation is a frequent medical requirement that can present challenges in the absence of a specific antidote.

Aims: To use the calibrated automated thrombogram (CAT) to assess suppression of thrombin generation by heparinoids and evaluate reversal by prothrombin complex concentrate (PCC) with or without protamine sulphate (PS) in an *in vitro* spiking study.

Methods: Thrombin generation (TGA) was measured as described by Hemker et al. Pooled plasma from healthy volunteers was spiked *in vitro* with a range of concentrations of unfractionated heparin (0–0.4 IU mL⁻¹), tinzaparin (0–1 IU mL⁻¹), or fondaparinux (0–2.5 µg mL⁻¹) and with a 4-factor PCC (0–0.9 IU mL⁻¹) with or without PS (2.5 µg mL⁻¹). Endogenous thrombin potential (ETP), peak thrombin, time to Peak (ttP) and lagtime (LT) were calculated.

Results: TGA dose-dependent increases and suppression were seen with PCC and heparinoids respectively. PCC did not result in any significant reversal of samples spiked with unfractionated heparin. Reversal was seen with PS. Tinzaparin samples demonstrated an approximately 50% increase in ETP with PCC compared to samples without at the lower end of concentrations tested although not at the higher end. When spiked with PCC and PS there was an increase in ETP to 50% above baseline. Fondaparinux samples also showed similar results with PCC but not PS. Patient anti-Xa samples (LMWH) spiked ex-vivo with PCC also demonstrated reversal of almost complete suppression to 50% of baseline ETP.

Conclusion: Suppression of thrombin generation by heparinoids can be partially reversed with PCC and, in the case of LMWH, synergistically with PS. However, spiking experiments do not reflect *in vivo* clearance and are disproportionately influenced by high molecular weight units with IIa activity. These assays need to be replicated on more patient samples.

Disclosure of Interest: None declared.

PO296-MON

Performance evaluation of a new assay† for the quantitative determination of both unfractionated heparin (UFH) and low molecular weight heparin (LMWH) using a hybrid calibration curve

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Background: Unfractionated (UF) and low molecular weight (LMW) heparin are frequently used as prophylactic and therapeutic anticoagulants in prevention and treatment of venous thromboembolism, in certain types of coronary artery syndrome and in thrombotic stroke. Therefore, it is suitable to monitor plasma level of the anticoagulants during treatment. While UFH considerably accelerates the inactivation of thrombin and coagulation factor Xa (Xa), LMWH has its main effect on Xa only.

Aims: The aim was to evaluate the performance of a new chromogenic Xa heparin assay† format, corresponding controls and calibrators. Using a hybrid curve it offers safety concerning sample mix-up in treatment with either UFH or LMWH.

Methods: We conducted a multi-center study with 314 samples of patients treated with either UFH or LMWH on the BCS® XP System (Siemens) and compared them with the HemosIL Heparin assay on the ACL TOP System (Instrumentation Laboratories, USA). Assay precision was determined by testing plasma pools and controls on 20 days in two runs with two single determinations ($20 \times 2 \times 2$ scheme). Additionally, we compared the assay with 147 samples on the Sysmex® CS-2100i* System (Sysmex Inc., Kobe, Japan) and BCS XP System.

Results: The correlation in the multi-center study showed a correlation coefficient of 0.982, slope of 1.10 and intercept of 0.01. For precision a within device CV $\leq 2.2\%$ was observed for all samples ≥ 0.5 IU mL⁻¹. For samples ≤ 0.5 IU mL⁻¹ SD was evaluated which was found to be < 0.03 IU mL⁻¹. Comparability of results between the BCS XP System and Sysmex CS-2100i* System was excellent (correlation coefficient ≥ 0.993 ; slope of 1.00; intercept 0.05).

Conclusion: The new assay is well suited for the measurement of both, UFH and LMWH. It demonstrated excellent precision, correlates well with another commercial available assay and shows excellent analyser comparability.

‡Under development. Product availability varies by country.

*Not available for sale in the U.S.

Disclosure of Interest: M. Merz Employee of: Siemens Healthcare Diagnostics Products GmbH, S. Lange Employee of: Siemens Healthcare Diagnostics Products GmbH, K. Madlener Grant/Research Support from: Siemens Healthcare Diagnostics Products GmbH.

PO297-MON

Strategies and outcomes of periprocedural bridging therapy with low-molecular-weight- heparin in patients with mechanical heart valves

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Background: Patients with mechanical heart valves (MHV) undergoing invasive procedures often receive periprocedural bridging with low-molecular-weight heparin (LMWH). The bridging strategies used in real-life and the predictors for bleeding and thrombosis are not well studied.

Aims: We aimed to evaluate the outcomes of the periprocedural bridging regimens in terms of perioperative bleeding and thromboembolic events, as well as the associated risk factors.

Methods: We retrospectively assessed patients with MHV that underwent an invasive procedure requiring vitamin K antagonist (VKA) interruption and LMWH bridging. Thromboembolic and bleeding events occurring up to 30 days after the procedures were recorded. Predictors of major bleeding events (MBEs) were analyzed with logistic regression.

Results: We evaluated 547 patients with MHV who underwent 275 procedures during a 6.5-year period. Bridging with LMWH was used in 185 procedures in a total of 117 patients. Combined pre- and postoperative bridging was the most frequently employed (63%). Doses of LMWH were prophylactic in 96 (52%) of the procedures and therapeutic in 89 (48%). The procedure-related bleeding risk was evaluated as high in 70 (38%) and low in 115 (62%) of the invasive procedures. There was a trend to more frequent use of prophylactic doses (61%) in high-risk surgery, and more therapeutic doses (53%) in low-risk ones. There were 36 bleeding episodes, 21 (11% of procedures) of which were classified as MBEs, but there were no thromboembolic events. Most MBEs ($n = 14$;

67%) occurred in surgeries with high bleeding risk. In the multivariate analysis, the bleeding risk of the surgery itself was the only independent predictor for MBEs.

Conclusion: For patients with MHV receiving perioperative bridging with LMWH, the major predictor for MBE is the bleeding risk of the surgery.

Disclosure of Interest: None declared.

PO298-MON

Biophysical characterization of the interaction between platelet factor 4 and synthetic low molecular weight heparins

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Background: Synthetic low molecular weight (ULMW) heparins are sulfated glycans that are clinically used to treat thrombotic disorders such as heparin-induced thrombocytopenia.

Aims: We characterize the interaction of platelet factor 4 (PF4) with different synthetic heparins of 6-, 8-, 10-, and 12-mers.

Methods: Circular dichroism spectroscopy was used to investigate changes in the secondary structure of PF4 induced by different synthetic heparins. The antigenicity of PF4/synthetic heparin complexes was assessed by enzyme immunoassay with sera of patients known to contain anti-PF4/heparin antibodies. The thermodynamics of the interaction between PF4 and synthetic heparins was determined by isothermal titration calorimetry. Single molecule force spectroscopy was used to compare the rupture forces between PF4 and different synthetic heparins.

Results: We found that all synthetic heparins induced conformational changes in PF4 (approx. 40% in anti-parallel β -sheet). Complexes formed by PF4 with heparins larger than 10-mers were proved to be antigenic, i.e. they bound PF4/heparin antibodies. For all investigated heparins, binding of heparins to PF4 resulted in heat release. However, longer synthetic heparins induced a higher heat release (negative change in enthalpy) compared with shorter synthetic heparins. Binding strength of PF4/heparin complexes increases with an increase in heparin length. A 12-mer heparin presenting an additional sulfate group interacts weaker with PF4 than a standard 12-mer heparin.

Conclusion: Our biophysical methods may be applied to understand the molecular interactions between PF4 and newly developed synthetic heparins.

Disclosure of Interest: None declared.

PO299-MON

Measurement of heparanase activity in plasma requires the use of sample antithrombin-depletion

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Background: Heparanase, expressed mainly by cancer cells, is correlated with increased tumor aggressiveness, metastasis, and angiogenesis due to degradation of Heparan sulfate proteoglycans (HSPGs) in the tumor environment. Therefore determination of its activity released in plasma from cancer patients is of great interest.

Aims: Determination of heparanase activity based on degradation of heparin and pentasaccharide in order to measure levels in plasma from cancer patients.

Methods: Techniques based on heparin degradation by purified heparanase at pH 5.5 (1) in purified system and in plasma: 1) degradation

of heparin or pentasaccharide by heparanase and evaluation of the residual activity by the anti-FXa activity using chromogenic substrate, 2) degradation of biotinylated heparin immobilized on streptavidin coated plates and evaluation of the residual biotin level remaining bound to the plate using streptavidin peroxidase. Experiments were done using plasma or antithrombin (AT) depleted plasma.

Results: In purified system, degradation of heparin, pentasaccharide, or biotinylated heparin were only evidenced at very high concentrations of heparanase (165 ng mL^{-1}), which are far higher than those reported in pathological situations. Lower concentrations of heparin or pentasaccharide in the assay to sensitize the assay cannot be used because the residual FXa activity is below the sensitivity of chromogenic method. In contrast, Heparin or its derivatives could not be degraded in plasma by heparanase, even at high concentrations. By using AT-depleted plasma, we showed that it was due to an unavailability of the site of heparin degradation by heparanase when heparin is bound to AT. So, the results were similar to those obtained in purified system.

Conclusion: Determination of heparanase activity in plasma has to be performed after depletion of AT of the sample to be tested. Optimization of the residual FXa measurement is requested to increase the sensitivity of heparanase assay.

Disclosure of Interest: None declared.

PO300-MON

Novel synthetic copolymers able to bind unfractionated and low molecular weight heparins

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Background: The wide use of various medical devices during intravascular or cardiac interventions increased the need for administration of heparins. Our group have already shown various cationic polysaccharides able to bind unfractionated heparin (UFH) *in vitro* (Kaminski et al., *J Med Chem*, 2010) and *in vivo* (Kalaska et al., *Eur J Pharmacol*, 2012, Kaminski et al. *MedChemComm*, 2014).

Aims: The aim of the present study was to obtain new synthetic heparin-binding copolymers (HBCs) and select the most potent inhibitors of both unfractionated and low molecular weight heparins (LMWH).

Methods: We synthesized a series of HBCs, then screened *in vitro* for the active ones and examined the most promising candidates *in vivo*. Binding of UFH, nadroparin or enoxaparin by HBCs was determined using Azure A assay and the dynamic light scattering measurement. Polymers were then administered to 23 male Wistar rats developing electrically induced arterial thrombosis according to the following treatment plan: UFH-3 min-HBC-7 min-electrical stimulus (1 mA)-10 min-thrombosis development-45 min-end. At the end thrombus weight, tail bleeding time, activated partial thromboplastin time, anti-factor Xa activity and blood count were estimated. Blood pressure was directly measured in 15 male Wistar rats to exclude poorly tolerated polymers. All the procedures involving animals and their care were approved by Local Ethical Committees.

Results: All tested polymers efficiently bound heparins. HBC1 exhibited the strongest ability to bind UFH, while the size of its complexes with UFH was the smallest. We found that only HBC1 reversed heparin antithrombotic effects in rats, without changing blood pressure, heart rate and platelet count. All HBCs restored the anti-factor Xa activity *in vivo* more efficiently than protamine or cationic dextran.

Conclusion: HBC1 could be a novel potent agent for the treatment of bleeding associated with UFH or LMWH therapy.

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Disclosure of Interest: None declared.

PO301-MON

Differences between normal and cancer cells in the bio-response to heparin

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Background: Heparan sulfate proteoglycans (HSPG) act as cell surface receptors which exert a variety of important roles in cell biology and human disease. Our previous work found that the heparin/HS interaction of human breast cancer cells exerts a pro-tumorigenic role and in light of a previous report that the expression of HSPGs is higher in tumours than in normal tissue, we considered whether this difference could elicit differences in the response of normal and cancer cells to heparin.

Aims: To investigate the bio-response to heparin treatment of normal and tumour cells.

Methods: Normal epithelial cells (MCF-12A, ME-16C, HEK293), and cancer epithelial cells (MCF-7, MDA-MB231, CSC, A549, HT1080) were treated with heparin for 2 days. Untreated cells were used as controls. The expression of miRNAs and pro-tumourigenic proteins and signalling activity were tested by RT-PCR and Western blot. Cell proliferation, apoptosis and cell cycle assays were also undertaken with heparin treated cells.

Results: The level of pro-tumorigenic marker proteins / molecules (MUC1 and p-Akt) was not significantly reduced by heparin treatment in normal cell lines, as was the case unlike with tumour cell lines. Differences were observed between the modulation of the expression of miRNA155, miRNA145 in normal compared to tumour cells. 12 cell cycle regulatory proteins were up-regulated by heparin treatment of normal cells (MCF-12a) while the majority were down-regulated by heparin treatment of breast cancer cells. Cell cycle assay showed that heparin treatment did not cause significant changes in S and G2-M phases in normal cells unlike that observed in tumour cells.

Conclusion: The biological response to heparin treatment of normal and tumour cells exhibit significant important differences.

Disclosure of Interest: None declared.

PO302-MON

Monitoring unfractionated heparin in children – APTT, anti-Xa and ACT in a randomized controlled trial of comparing two dose protocols

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Background: Monitoring of unfractionated heparin (UFH) in children is crucial for prevention of over- or undercoagulation. However, recommendations for monitoring are extrapolated from adult studies and their significance for children is increasingly questioned.

Aims: To i) investigate the relationship between UFH dose and its anticoagulant effect as assessed by three commonly-used assays (anti-Xa, APTT, ACT), ii) assess factors determining the UFH effect and iii) determine the agreement between the respective assays.

Methods: Randomized controlled trial comparing high-dose UFH (100 units kg^{-1} bolus, followed by 20 units $\text{kg}^{-1} \text{ h}^{-1}$ continuous infusion) versus low-dose UFH (50 units kg^{-1} bolus) during cardiac catheterization.

terization (CC) in children. Blood samples were drawn prior to UFH administration, after 30 and 60 min and then hourly for the duration of CC.

Results: Four-hundred-two bloods samples of 149 patients were evaluable. Median age was 5.5 years (0.01; 18), and 29 (19%) patients were infants. Linear mixed model demonstrated a significant relation between UFH dose and the respective UFH effect as assessed by anti-Xa, APTT and ACT. Besides UFH dose, higher age and higher anti-thrombin at baseline were associated with higher anti-Xa-levels. Higher Age and APTT at baseline had a significant influence on APTT after UFH administration. For the ACT, only ACT at baseline was a significant factor determining the UFH effect. Notably, the agreement between the three assays was very low.

Conclusion: All three assays demonstrated a significant dose-effect relation of UFH, however with only little agreement between them. The ACT was the only assay not influenced by patients' age. Our results suggest that UFH monitoring in children must always take into account the patients' age and that titrating UFH dose to achieve a certain therapeutic range must be reconsidered cautiously.

Disclosure of Interest: None declared.

PO303-MON

Ovine mucosal heparin and enoxaparin are biosimilar to their porcine counterparts

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Background: Heparins are a widely used anticoagulants which can be derived from such mammalian sources such as porcine, bovine and ovine mucosa. Ovine mucosal tissue provides an alternate source to manufacture heparin and enoxaparin.

Aims: These studies are designed to compare ovine heparin and enoxaparin with their porcine counterparts.

Methods: Porcine heparin and enoxaparin were obtained commercially. Ovine heparin and enoxaparin were obtained from Ronnsi Pharma Co., Suzhou, China. The molecular weight profile was investigated using HPLC methods. The anticoagulant activities were compared utilizing ACT, aPTT, Heptest, and Calcium Thrombin Time. The anti-Xa and anti-IIa were measured using chromogenic substrate methods. Protamine neutralization studies were also carried out in various test. Thrombin generation inhibition studies were carried out using a floremetric method. Heparinase digestion profile was investigated using oligosaccharide mapping.

Results: The molecular weight profile of the porcine heparin (16.6 kDa) was comparable with the ovine heparin (17.1 kDa). The molecular weight profiles of the porcine enoxaparin (4.0 kDa) was almost identical to the ovine mucosal enoxaparin (3.9 kDa). On a gravimetric basis, in all clotting tests (ACT, aPTT, Heptest and Thrombin Time) and chromogenic assays (anti-Xa and anti-IIa) heparin and enoxaparin from porcine and ovine sources exhibited similar profiles. Both groups of heparins were found to be equally neutralized by a fixed concentration of protamine sulfate. Thrombin generation profile for bovine and porcine enoxaparin were comparable. Heparinase treatment resulted in similar oligosaccharides profiles.

Conclusion: The molecular weight profile between the groups of heparins and enoxaparins is similar. The anticoagulant and anti-protease properties of ovine derived heparin and enoxaparin are also similar to porcine derived sources. Ovine derived heparin and enoxaparin are a suitable alternative to the currently overused porcine mucosal heparins and enoxaparins.

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PO304-MON

The efficacy and safety of low-molecular-weight heparin thromboprophylaxis in critically ill adult patients: a systematic review and meta-analysis

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Background: Critically ill patients have an increased risk of venous thromboembolism due to several additive risk factors. ACCP guidelines recommend the use of low-molecular-weight heparin (LMWH) or unfractionated heparin (UFH) to prevent VTE in critically ill patients. The data supporting their prophylactic effect are mainly based on studies in medical and surgical ward patients.

Aims: The aim of this systematic review and meta-analysis was to synthesize the literature on the efficacy and safety of LMWHs as thromboprophylactic agents in critically ill patients and assessment of LMWH prophylaxis with plasma anti-FXa level monitoring.

Methods: Medline, Scopus, the Cochrane Library and the ClinicalTrials.com database were used. Prospective randomized controlled trials (RCTs) of critically ill adult patients that compared any LMWH thromboprophylaxis either to unfractionated heparin (UFH), mechanical prophylaxis, novel oral anticoagulants (NOACs) or placebo were included in the meta-analysis.

Results: Nine RCTs involving 7258 patients were included in the meta-analysis. The risk of proximal DVT during LMWH thromboprophylaxis remained unchanged compared to UFH (risk ratio 0.92, 95% confidence interval 0.73–1.15) or to placebo (RR 0.76, 0.49–1.19). The risk of all-cause mortality was 0.93 (0.82–1.04) compared to UFH, and 0.859 (0.73–1.02) compared to placebo. The risk of PE was reduced compared to UFH (RR 0.63, 0.39–0.997). LMWH did not increase the risk of major bleeding when compared to UFH (RR 0.97, 0.75–1.26) or to placebo (RR 2.26, 0.90–6.71). In the 15 studies included with reported anti-FXa levels, the median peak anti-FXa level varied between <0.1–0.34 IU mL⁻¹.

Conclusion: Compared to UFH, LMWHs seem to be equally as efficient and safe, and LMWHs could reduce the risk of PE. Compared to placebo, the risk of all DVT is reduced without increasing the risk of major bleeding. Data on mechanical prophylaxis remain insufficient. No conclusions can be drawn from the targeted anti-FXa level in critically ill patients.

Disclosure of Interest: None declared.

PO305-MON

Comparative anticoagulant and anti-inflammatory effects of sulodexide and enoxaparin in citrated whole blood

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Background: Sulodexide represent a novel antithrombotic agent with multiple sites of actions on humoral and cellular processes. Sulodexide represent of mixture of fast mobilizing heparin 80% and 20% derma-

tan sulfate, with an average MW of 20.8 kDa. This agent has been used in various vascular indications such as chronic venous disorders. Unlike low molecular weight heparin (LMWH), this agent can also be used orally.

Aims: The purpose of this study was to compare the anticoagulant and anti-inflammatory effects of sulodexide with a LMWH, enoxaparin.

Methods: In order to compare the anticoagulant and anti-inflammatory effects of sulodexide and enoxaparin, each of these agents were supplemented to citrated whole blood ($n = 10$) at concentrations of 0–25 $\mu\text{g mL}^{-1}$ and incubated for varying amounts of time. Such coagulation tests as ACT, PT, APTT, Heptest, and Thrombin time (TT) were measured in the whole blood and retrieved plasma. In addition, thrombin generation inhibition studies were carried out. To test the anti-inflammatory effects, bacterial lipopolysaccharide stimulated whole blood samples were compared utilizing a cytokine biochip array to measure cytokine and growth factor arrays such as IL-1 α , IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN, EGF, MCP-1, TNF α .

Results: In comparison to enoxaparin and sulodexide produced a stronger anticoagulant effect in the PT, APTT and TT assays ($P < 0.05$). In the Heptest assay, sulodexide produced anticoagulant effects which were similar to enoxaparin. Sulodexide produced stronger inhibition of thrombin generation ($P < 0.05$). In the cytokine biochip array, sulodexide produced stronger suppression of IL-6, TNF α and MCP-1 generation, in contrast to enoxaparin ($P < 0.05$).

Conclusion: These results strongly suggest that sulodexide is a relatively stronger anticoagulant and anti-inflammatory agent than enoxaparin. The dual anti-thrombotic and anti-inflammatory actions of this agent contribute to its clinical efficacy in vascular diseases.

Disclosure of Interest: None declared.

PO306-MON

In vitro evaluation of novel synthetic heparan sulfates mimetics to prevent xenogeneic related-thrombosis

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Background: Xenotransplantation aims to develop methods to use pig organs in place of human organs. During the past decade, significant advances were made to control antibody and complement mediated xenograft rejection due to the generation of genetically modified pigs. However, xenogeneic organs are still injured and consumptive coagulopathy develops in xenograft recipients. Inflammation and coagulation cascade activation have been identified as important therapeutic targets.

Aims: Glycobiologists and transplant immunologists collaborated to evaluate novel heparan sulfate (HS) mimetics targeting factor Xa, XIa and/or thrombin to prevent xenogeneic coagulation and platelet aggregation.

Methods: Synthetic HS mimetics were screened in a xenogeneic coagulation assay (human plasma incubated with porcine aortic endothelial cells (pAEC)) (100 μM). Selected mimetics were further characterized for their ability to inhibit thrombin- and pAEC-induced human platelet aggregation and transgenic human thrombomodulin-dependent activation of Protein C. Therapeutic dosing range in whole blood was estimated by thromboelastography (TEG) and Activated Clotting Time (ACT).

Results: Clotting score was significantly decreased by heparin (4 IU mL^{-1} , score 0 ± 0.3) and Hirulog (200 ng mL^{-1} , 0.03 ± 0.3)

compared to no treatment (2.6 ± 0.7 , $P < 0.001$). 18 of the 34 HS mimetics inhibited clotting to variable levels, 5 of which were comparable to heparin and hirulog. Heparin and two HS mimetics inhibited thrombin- and pAEC-induced platelet aggregation more potently than low molecular weight heparin. Four HS mimetics prevented blood clotting to similar extent as heparin (1–3 IU mL^{-1}) (ACT >300 sec., reaction time $R > 200$ min, maximum amplitude MA <10 mm) at concentrations of 0.2–1 mM.

Conclusion: HS mimetics show promise to control the coagulation dysregulation associated with xenotransplantation. Future work will evaluate the best HS mimetics under flow conditions and in ex-vivo lung perfusion experiments, as well as explore more complex synthetic molecules.

Disclosure of Interest: None declared.

PO307-MON

Argatroban vs. fondaparinux in heparin-induced thrombocytopenia patients: a response comparative study

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Background: Alternative non-heparin anticoagulants such as argatroban and fondaparinux are used in the management of patients with heparin-induced thrombocytopenia (HIT).

Aims: To evaluate the comparative response of argatroban vs. fondaparinux for patients with HIT.

Methods: A retrospective study was conducted at King Abdulaziz Medical Center a university teaching hospital on patients diagnosed with HIT between June 2013 and December 2014. Clinical and laboratory findings were used to confirm the positive HIT cases.

Results: Thirty three patients were identified as a HIT positive by enzyme-linked immunosorbent assay. Argatroban was given to 21 patients and fondaparinux was administered in 12 subjects. The main demographic characteristics of the argatroban and fondaparinux were comparable and no statistical significant differences. The mean time (in days) for platelets recovery was $3.5 (\pm 1.8)$ for patients who received argatroban compared with $3.7 (\pm 1.7)$ patients received fondaparinux ($P = 0.843$). The pre-test probability 4Ts scoring system and complications were similar for both group ($P > 0.05$).

Conclusion: Our findings suggest that argatroban and fondaparinux are comparable non-heparin anticoagulants for HIT management

Disclosure of Interest: None declared.

PO308-MON

Anticoagulant and fibrinolytic effects of complexes of dipeptide Pro-Gly with low-molecular-weight heparins animal and plant origin

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Background: It has been found that complex compounds of unfractionated heparin with proline- and glycine-containing (glyproline) peptides possessed fibrinolytic, anticoagulant and antithrombotic activities. In our previous experiments we showed that addition of the complexes of dipeptide Pro-Gly with low-molecular-weight heparin and plant heparin-like agent to rat blood plasma caused the increase of non-enzymatic fibrinolytic and anticoagulant activities thus inhibited stabilization of fibrin *in vitro*.

Aims: study the influence of complex compounds of dipeptide Pro-Gly with LMWH (LMWH-PG) and plant heparin-like agent (PH-PG) on parameters of plasma hemostasis *in vivo*.

Methods: A low-molecular heparin-like component was released from plant (*Paeonia suffruticosa*). Complexes LMWH-PG and PH-PG (1:1 w/w ratio) were synthesized by our methods. The experiments were carried out on health white male rats. All experiments were performed in accordance with the NIH Guide for Care and Use of Laboratory Animals. Blood samples were taken after 10 min after the intravenous injection of complexes (1 mg kg⁻¹ body weight). Anticoagulation activity of blood plasma was detected by standard clotting method (APTT-test). Non-enzymatic fibrinolytic (NF) and total fibrinolytic (FA) activities were estimated by non-stabilized fibrin plate's method.

Results: It was established that injection of LMWH-PG led to a marked increase of anticoagulant (in 2 times from control level), FA (in 1.7 times) and NF (in 1.52 times) activities of blood. Administration of complex PH-PG caused a more significant increase of anticoagulant and fibrinolytic properties of blood (APTT in 3.2 times, FA in 1.92 times, NF in 2 times from control level).

Conclusion: Thus, injection of complex compounds to health rats leads to increase of anticoagulant and fibrinolytic activities of blood plasma. The identity of the action on haemostatic system of complexes with heparin of animal and plant origin was found with PH-PG complex was more effective than LMWH-PG.

Disclosure of Interest: None declared.

Immune thrombocytopenia (ITP) I

PO309-MON

Usage of elthrombopag in a 9 year old Syrian patient with immune thrombocytopenic purpura and familial deafness-first pediatric experience in Turkey

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Background: Thrombopoetin receptor agonists (romiplostim and elthrombopag) have recently been licensed for the treatment of thrombocytopenia in patients with chronic immune thrombocytopenia (ITP) who do not respond to conventional treatment like corticosteroids (CS), immunoglobulins (IVIG) and splenectomy.

Aims: Here, we describe a 9 year old Syrian patient with familial deafness and chronic ITP unresponsive to CS (Methyl prednisolone and dexamethasone), IVIG, splenectomy, cyclosporin, rituximab and oseltamivir phosphate. As first pediatric use in Turkey, elthrombopag was tried as a salvage therapy.

Methods: A 9 year old Syrian refugee patient was admitted with GI bleeding. He was deaf and unable to talk since he was born and there were three more patients with deafness in the family. He was splenectomized at the age of six was steroid dependent. MYH9 disorder was excluded with normal myosin IIA staining. Bone marrow examination was consistent with ITP.

Results: He was unresponsive to IVIG, danazol, vincristin, dexamethasone plus cyclosporin, 4 weeks treatment of rituximab, anti-D and oseltamivir phosphate. A slight and transient increase could only be achieved with mega dose MPZ. The platelet levels reached to a maximum of $10 \times 10^9 \text{ L}^{-1}$ and he had several episodes of GI and severe nose bleedings requiring transfusion. Elthrombopag $2 \times 50 \text{ mg}$ per day in accordance with MPZ was started. Elthrombopag could be used for only 2 weeks, unfortunately no increase in platelet count could be

achieved (maximum $8 \times 10^9 \text{ L}^{-1}$) and the patient was lost because of massive GI bleeding.

Conclusion: Despite several treatment modalities in chronic ITP, some patients are unresponsive to all of them. Although we tried elthrombopag as a salvage therapy, the patient was lost because of massive bleeding. We suppose ethnic differences in immune system function may also have played a role in response to elthrombopag in our patient. Further pediatric studies to investigate elthrombopag use in chronic ITP are warranted.

Disclosure of Interest: None declared.

PO310-MON

Acute immune thrombocytopenic purpura following combined vaccine

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Background: Individual case reports have subsequently been published describing ITP after vaccination against measles, mumps and rubella; smallpox; diphtheria- tetanus- pertussis; hepatitis; and influenza and pneumococcal infection. Most of them are related to MMR immunisation in childhood.

Aims: Herein we report a case with acute immune thrombocytopenic purpura that were developed after DTaP-IPV-Hib combined vaccine (diphtheria/tetanus/acellular pertussis, *inactivated poliomyelitis*, Haemophilus influenzae type b) and conjugate pneumococcal vaccines.

Methods: Herein we report a case with acute immune thrombocytopenic purpura that were developed after vaccination.

Results: A 5-months-old male was brought to emergency department with the complaint of petechial rash, for 1 days, begun 3 days after an DTaP-IPV-Hib combined vaccine and PCV. The physical examination of patient was normal except the presence of bleeding multiple petechiae rash on lower and upper extremities and trunk. The laboratory examinations included complete blood count, prothrombin time, partial thromboplastin time, serum electrolytes, liver and renal function test, brucella and salmonella serology were found to be normal except severe thrombocytopenia. (platelet count 1000 mm^{-3}). Considering the complete clinical examination and investigations, a final diagnosis of ITP was made. Normal thrombocyte count was achieved on fifth day of treatment on which thrombocyte count was measured as $150\,000 \text{ mm}^{-3}$.

Conclusion: Our case showed that acute ITP might be seen after DTaP-IPV-Hib combined vaccine and PCV. Therefore, we suggest that aside from a recent infectious disease and DTaP-IPV-Hib combined vaccine and PCV should also be inquired in children with acute ITP

Disclosure of Interest: None declared.

PO311-MON

Thrombopoietin receptor agonists do not cause coagulation activation: in patients with immune thrombocytopenia

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Background: Thrombopoietin receptor agonists (TPO) are effective treatment in immune thrombocytopenia (ITP). The rate of thrombotic events was reported in up to 6% of patients in TPO-agent trials raising concerns of coagulation activation.

Aims: To evaluate the effect of TPO-agents on procoagulant status in ITP patients.

Methods: The study comprised 2 ITP cohorts. Cohort 1 ($n = 26$) with sequential blood samples at 0, 2, 6 and 12 weeks. Cohort 2 ($n = 18$) patients with single samples treated for >1 year with TPO. Markers of coagulation and fibrinolysis including endogenous thrombin potential (ETP) by calibrated automated thrombography (CAT) assay, prothrombin fragments 1 + 2 (F_{1+2}), plasminogen activator inhibitor-1 (PAI-1) activity, and D-dimer were assayed in all samples.

Results: Mean age was 55 years (32% males). 19 patients were treated with romiplostim, 22 with eltrombopag and 3 with avatrombopag. Median values of D-dimer at 0, 2, 6, 12 weeks and >1 year were: 413, 537, 477, 455, and 441 ng mL⁻¹ (cut-off <500 ng mL⁻¹), for F_{1+2} : 298, 275, 280, 308, and 420 pmol L⁻¹ (reference interval 69 - 229 pmol L⁻¹), for PAI-1: 1.5, 1.7, 1.2, 1.0, and 1.0 ng mL⁻¹ (cut-off <5 ng mL⁻¹), and for ETP (normalized against pooled normal plasma): 86, 88, 91, 87, and 96 (reference interval 75–138), and for platelet counts: 19, 116, 78, 56, 106·10⁹ L⁻¹. Significant increase in platelet counts was found after 2, 6, and 12 weeks and after >1 year of TPO treatment, but no significant changes were found in D-dimer, F_{1+2} , PAI-1 or thrombin generation (ETP), except for ETP 6 weeks after treatment compared to before treatment ($P = 0.042$). No significant differences in any parameters were found between pretreatment samples in cohort 1 and after long-term treatment in cohort 2 (>1 year).

Conclusion: Coagulation is not activated by TPO-agents. Previous studies have shown that neither platelets are activated. This indicates that other mechanisms are affected by TPO to increase the risk of thromboembolism.

Disclosure of Interest: None declared.

PO312-MON

Treatment-related adverse event burden in immune thrombocytopenic purpura (ITP)

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Background: Real-world evidence on the safety profile and costs associated with ITP treatment in adults is lacking.

Aims: To quantify and compare adverse event (AE) rates and related costs in ITP patients treated with anti-D (AD), intravenous immunoglobulin (IVIG), rituximab (RIT; off-label), romiplostim (ROM) or eltrombopag (ELT).

Methods: Retrospective claims analysis was conducted in the IMS PharMetrics Plus database. Included patients were ≥18 with diagnosis of ITP (2007–2012), 1 year of continuous enrollment (CE), and an ITP-related claim for AD, IVIG, ROM, or ELT; a 3-year CE subset was examined for RIT. Relevant AEs were informed by package inserts and identified by ICD9 code during active treatment, defined from 1st claim of each drug to a pre-defined treatment gap or end of study period. Descriptive statistics were reported with Wilcoxon rank-sum significance tests ($P < 0.05$).

Results: A total of 2518 adult patients (AD: 364, IVIG: 879, RIT: 1,025, ROM: 175, ELT: 75) were identified (mean age 50.8 (±16.3 years), 55.8% male). Of all patients, 22.8% experienced any AE. Significantly fewer AD patients had any AE (13.8% vs. IVIG: 21.1%, RIT: 29.4%, ROM: 28.1%, ELT: 22.4%). Nausea/vomiting and arthralgia/musculoskeletal pain were most common across treatments, followed by headache and upper respiratory infection. AD had significantly fewer headaches than IVIG, less nausea than RIT and ELT, and fewer infections and arthralgia/pain vs. other treatments. Hemolytic events did not differ significantly across treatments. Most costly AEs were urinary infection, aseptic meningitis, and fever (\$5000 + /case); headache, nasal congestion, and hemolytic event were \$4000–5000/case. Cost per AE did not differ by treatment.

Conclusion: Although lower than trial-based AE rates, claims for ITP treatment-related AEs are common, with higher numbers for RIT and lower numbers for AD. This disparity suggests a possible differential cost burden overall that future analysis should explore.

Disclosure of Interest: P. Donga Employee of: IMS Health, S. P. Bilir Employee of: IMS Health, J. Munakata Employee of: IMS Health, G. Little Employee of: Emergent BioSolutions, T. Babinchak Employee of: Emergent BioSolutions.

PO313-MON

Hashimoto's thyroiditis in children with chronic idiopathic thrombocytopenia

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Background: The central process of the pathogenesis of the autoimmune disorders is disrupted self-tolerance. This point explains why patients with one autoimmune disease are prone to develop other disorders of autoimmune nature. Immune thrombocytopenic purpura (ITP) is one of the most common autoimmune disease during childhood.

Aims: To evaluate the incidence and clinical features of Hashimoto's thyroiditis in children with chronic ITP.

Methods: Fifty-four children with a median age of 11 years who were diagnosed as chronic ITP according to IWG criteria were enrolled in this study. Thyroid functions [(fT4: 0.88–1.63 ng dL⁻¹), (TSH: 0.51 - 4.3 IU L⁻¹)] and anti-thyroid antibodies [Anti-TPO (anti-thyroid peroxidase) and anti-TG (anti-thyroglobulin)] were evaluated. Hashimoto's thyroiditis was diagnosed with the elevation of at least one of the both antibodies, anti-TG (> 64 IU L⁻¹) and anti-TPO (> 26 IU L⁻¹). Besides; we analyzed the thyroid functions according to serum TSH levels.

Results: The median thrombocyte level at the last examination of our patients was 2.7×10^9 L⁻¹ ($1.55\text{--}98 \times 10^9$ L⁻¹). Median fT4 and TSH levels were 1.15 ng dL⁻¹ and 2.3 IU L⁻¹, respectively.

Median anti-TG and anti-TPO levels were also 6.11 IU L⁻¹ (0–621 IU L⁻¹) and 11.9 IU L⁻¹ (0.1–216 IU L⁻¹), respectively. Isolated anti-TG elevation was seen in three patients. The other 3 patients have elevated anti-TPO levels whereas only one patient had both elevated anti-TG and anti-TPO. It has been shown that seven of our patients

had Hashimoto's thyroiditis (12.9%). Four of them had thyroid ultrasonography pointing diffuse heterogeneity without nodule. Only 4 of them had compensated hypothyroidism.

Conclusion: The most common cause of acquired hypothyroidism is Hashimoto's thyroiditis in children. In a previous survey, 6.3% and 4.8% of adolescents had positive anti-Tg and anti-TPO antibodies, respectively. In our study, we shown that Hashimoto's thyroiditis is more common in children with ITP than healthy adolescents.

Disclosure of Interest: None declared.

PO314-MON

Serial mean platelet volumes in immune thrombocytopenia

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Background: Elevated mean platelet (PLT) volume (MPV) is associated with immune thrombocytopenia (ITP) but not with hypoproliferative thrombocytopenia. The time course of the MPV in adult ITP is not known.

Aims: To characterize the time course of the MPV in adult patients hospitalized with ITP.

Methods: Institutional Review Board approval was obtained. The ITP ICD-9 code 287.31 was used to identify all patients with acute ITP admitted to Mount-Sinai Roosevelt and Mount-Sinai St. Luke's Hospitals between July 2012 and June 2013. A comparison group included 4 patients with chemotherapy-induced thrombocytopenia admitted to the hematology-oncology service between December 2013 and April 2014. Daily PLT counts and MPV's were recorded. The normal ranges for PLT count and MPV at our institution are 150 000–450 000 μL^{-1} and 6.5–11.3 fL respectively. Linear regression was utilized to study the relationship between PLT count and MPV.

Results: Ten admissions in 8 patients with ITP met criteria for enrollment. The MPV was normal in 7 cases (Group 1) and elevated in 3 cases (Group 2) on admission. Mean PLT count on admission was similar between the 2 groups: 6700 μL^{-1} and 8670 μL^{-1} respectively, $P > 0.05$. Steroids were administered to all patients. The MPV rose in all cases during hospitalization and correlated with increase in PLT count ($r = 0.464$, $P < 0.01$). None of the patients with chemotherapy-induced thrombocytopenia manifested an increase in MPV during PLT nadir or PLT recovery.

Conclusion: The MPV is usually normal on admission for ITP then, in steroid-treated patients, increases linearly with PLT count during recovery. It is possible that the MPV time course in this study reflects steroid-inhibition of phagocytosis, allowing for young platelets to circulate.

Disclosure of Interest: None declared.

PO315-MON

Rituximab experience in childhood immune thrombocytopenic purpura from a tertiary center in Turkey

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Background: Corticosteroids and intravenous immunoglobulin (IVIg) are standart treatment methods for childhood immune thrombocytopenic purpura (ITP). In case of failure of these drugs, rituximab (RTX) should be used as 2nd line drug for treatment. It depletes B cells by binding to surface CD20 antigens leading to removal of

autoreactive B cell clones. It has been reported that its initial response rate in primary ITP was 60–68% whereas 5-year response rate was 26%.

Aims: To evaluate the response to RTX therapy in childhood ITP.

Methods: The response and side effects of RTX therapy used in 8 childhood ITP patients (4 females) were assessed retrospectively. All the patients had RTX as the 2nd line drug after steroid and/or IVIg therapy. Hepatitis B serology was analyzed before treatment in all patients. No patient had splenectomy. RTX was given in a dose of 375 mg m^{-2} per week for 4 weeks. One patient developed anaphylactic reaction and RTX was ceased immediately. It was not tried for the same patient again. He was excluded from the evaluation. Thrombocyte count between 50–100 $\times 10^9 \text{ L}^{-1}$ was accepted as partial response (PR) whereas the levels above 100 $\times 10^9 \text{ L}^{-1}$ was accepted as complete response (CR).

Results: Mean age of the patients at the time of treatment was 128 months (range: 50–180 months). Mean duration to time of RTX therapy was 35 months (range: 2–103 months). Median thrombocyte count before RTX was 8.5 $\times 10^9 \text{ L}^{-1}$. Median duration to response was 4 months (3–12 months). CR and PR were achieved in 3 and 1 patients, respectively. No response was obtained in 3 patients. The median platelet count in the responsive patients was 114 $\times 10^9 \text{ L}^{-1}$. No infection, thrombosis or side effect was noted in our patients.

Conclusion: Concerning infections, RTX should be used as 2 nd line therapy safely. But, the longer duration of follow up is needed to evaluate the efficacy of RTX.

Disclosure of Interest: None declared.

PO316-MON

ITP with thyroid dysfunction: not merely an association- prospective analysis

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Background: Immune thrombocytopenia is characterized by immune mediated platelet destruction and suppressed platelet production leading to thrombocytopenia and bleeding. Primary ITP is characterized by isolated thrombocytopenia without other causes. Secondary ITP could be associated with infection with *Helicobacter pylori*, HIV, lupus, thyroid dysfunction etc. Mild-moderate thrombocytopenia common with hyperthyroidism and hypothyroidism is due to reduced non immune platelet survival and impaired production respectively.

Aims: To study prevalence of thyroid dysfunction in ITP.

Methods: This prospective study included 146 patients with age > 12 years, diagnosed as ITP. Serum T4 and TSH levels and anti thyroid peroxidase antibodies were tested by electrochemiluminescence test. All patients were treated as per standard guidelines and those with treatable secondary causes like H. Pylori, thyroid dysfunction were also given specific treatment.

Results: Most of our patients were females (67%), having either persistent or chronic ITP (85%). Abnormal T4 levels were present in 29 patients (27 hypothyroid and 2 hyperthyroid). Total 26 patients had abnormal TSH. Antithyroid peroxidase antibodies were demonstrated in 9 patients (31%) with abnormal T4 (P value 0.038) and 14 patients (54%) with raised TSH (P value 0.021). Hypothyroidism was significantly associated with presence of anti-nuclear antibodies (P value 0.011). Thyroid dysfunction was associated with chronic or persistent ITP but not with response to treatment.

Conclusion: Thyroid dysfunction is common in ITP ($n = 43$, 29%). Raised T4 can be due to effect of estrogen containing pills prescribed frequently to females with menorrhagia. Disproportionately low TSH can be seen with patients on glucocorticoids. Screening with TSH alone is likely to be insensitive and we suggest combined T4 and TSH for this purpose. Presence of anti-thyroid antibodies suggests autoim-

immune nature of disease. Results of treatment of thyroid disease and impact on platelet count are variable.

Disclosure of Interest: None declared.

PO317-MON

Levels of cytokines secreted by T lymphocyte and the relationship with prognosis in children immune thrombocytopenia

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Background: Many studies discovered that thrombocytopenia in ITP was not merely mediated by auto-antibodies, there might as well be abnormalities in cellular immunity, including disturbance of TH1/TH2 balance. Nevertheless, it remains unclear whether the levels of TH1 and TH2 cytokines influence the prognosis of children ITP.

Aims: Our study was designed to investigate the relationship between T lymphocytes' cytokines and prognosis in children immune thrombocytopenia (ITP).

Methods: We enrolled children ITP patients at the onset of their disease without any treatment from our department between December 2011 and March 2013. Blood samples measurement by cytometric bead array included IL-2, IL-4, IL-6, IL-10, TNF, IFN and IL-17. After following up for 1 year, all the enrolled patients were divided into 3 groups according to the remission time: Newly-diagnosed ITP Group, Persistent ITP Group and Chronic ITP Group. We compared levels of different cytokines among 3 groups and found risk factors for progression of the disease.

Results: There were 54 children ITP patients enrolled, including 41 (76%) formed Newly-diagnosed ITP Group, 6 (11%) formed Persistent ITP Group, 7 (13%) formed Chronic ITP Group. We found medians of TNF, IL-10 and IL-17 measured in Persistent ITP Group were the highest and IL-4, IL-6 was the highest in Chronic ITP Group. IL-2/IL-4 was the lowest in Chronic ITP Group, which indicated that Th2 polarization. However, levels of cytokines we detected were not significantly different among 3 groups with different prognosis. Besides, we found TNF might be one of the factors participating in the prolongation of the disease to more than 3 months ($P < 0.05$, OR 1.087), while IFN might be an indicator of a better prognosis ($P < 0.05$, OR 0.911).

Conclusion: In this study, we found Th2 polarization at the onset of disease will probably have a deferred course and worse prognosis. TNF and IFN might be indicators which could predict ultimate duration of children ITP.

Disclosure of Interest: None declared.

*On behalf of The first author.

PO318-MON

Elevated expression of IL-37 in patients with immune thrombocytopenia

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Background: Immune thrombocytopenia (ITP) is an autoimmune hemorrhagic disease. The pathogenesis is complex and may involve many

cytokines. As a new anti-inflammatory cytokine of the IL-1 family, IL-37 was discussed in many immune-mediated diseases.

Aims: This study was aimed at clarifying the role of IL-37 in the patients with immune thrombocytopenia (ITP).

Methods: In this study, we have measured plasma IL-37, IL-18, TGF- β and IFN- γ levels by ELISA, and tested the expression of mRNA by RT-PCR in 20 active ITP patients, 20 remission patients and 20 healthy controls.

Results: The plasma levels of IL-37 in active patients (144.54 ± 63.98 pg mL⁻¹) were significant higher than the controls (49.34 ± 37.26 pg mL⁻¹, $P < 0.05$), and remission patients (96.03 ± 65.14 pg mL⁻¹, $P < 0.05$). The plasma levels of IL-18 and IFN- γ in active patients were significantly increased compared with healthy controls (511.84 ± 319.95 vs. 284.22 ± 156.41 pg mL⁻¹, $P < 0.05$; 348.29 ± 145.06 vs. 225.83 ± 78.41 pg mL⁻¹, $P < 0.05$), with remission patients (511.84 ± 319.95 vs. 282.04 ± 183.03 pg mL⁻¹, $P < 0.05$; 348.29 ± 145.06 vs. 162.94 ± 104.33 pg mL⁻¹, $P < 0.05$), while plasma TGF- β in the active patients was lower than the healthy peoples (7.44 ± 5.59 vs. 23.91 ± 5.14 mg mL⁻¹, $P < 0.05$), and remission patients (7.44 ± 5.59 vs. 11.13 ± 4.43 mg mL⁻¹, $P < 0.05$). Compared to the healthy peoples, the mRNA expression of IL-37, IL-18 and TGF- β in active patients were significant difference ($P < 0.05$), but there was no significant difference observed for all of the four kinds of cytokines gene expressions in remission patients compared to controls.

Conclusion: The expression of IL-37 is fairly low in healthy peoples, while IL-37 is up-regulated in active ITP patients. After treatment, IL-37 decreased significantly in remission patients. Cytokine IL-18, TGF- β and IFN- γ also involved with this disease. The results indicate that IL-37 is associated with the complex pathogenesis in ITP, and the IL-37 may become a potential therapeutic target for ITP.

Disclosure of Interest: None declared.

PO319-MON

Analysis of histocompatibility locus antigen (HLA)-G and immunoglobulin-like transcripts (ilts) on patients with immune thrombocytopenia

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Background: Histocompatibility locus antigen (HLA)-G is a group of non-classical HLA class-I molecule. HLA-G exerts suppressive effects via interacting with its inhibitory receptors.

Aims: Our study aimed to investigate the role of HLA-G and its inhibitory receptors in immune thrombocytopenia.

Methods: The expression of HLA-G and its receptors ILT2 and ILT4 in peripheral blood mononuclear cells (PBMCs) from patients with ITP, and the effect of rhHLA-G protein in correcting the aberrant expression and function were evaluated.

Results: A significantly lower level of sHLA-G in the plasma from ITP patients positive for either anti-GPIIb/IIIa or anti-GPIb autoantibody was found in comparison to healthy control. However, no difference was observed between the autoantibody-negative patients and healthy control. The platelet count was positively correlated with the concentration of HLA-G in ITP patients. Compared with healthy control, HLA-G and ILT2 in CD4⁺ and CD19⁺ cells, and HLA-G and ILT4 in CD14⁺ were significantly lower in ITP patients. The rhHLA-G protein could up-regulate the expression of ILT2 in CD4⁺, CD19⁺ cells and ILT4 in CD14⁺ cells. The apoptosis of platelets co-cultured with rhHLA-G-modulated autologous PBMCs was significantly reduced in comparison with unmodulated PBMCs. There was no difference in the proportion of regulatory T cells with or without the treatment of rhHLA-G. The expression of CD86 and CD80 in dendritic cells (DCs)

were found to be decreased in the presence of rhHLA-G in both ITP patients and healthy control.

Conclusion: Our study demonstrated that the rhHLA-G could increase the cell-mediated platelet destruction and inhibit the maturation of DCs in patients with ITP, thus propose new therapeutic strategies for ITP.

Disclosure of Interest: None declared.

PO320-MON

An increased major lipid raft protein raftlin associated with b cells signaling in patients with immune thrombocytopenia

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Background: Raftlin, termed as raft-linking protein, is identified from raft-associated proteins and necessary for the integrity of the lipid raft and lymphocytes signal transduction. It was reported that the raftlin enhanced Th17-mediated autoimmune responses in mice. Disruption of raftlin gene in DT40 B cells resulted in the proliferation reduction and BCR signaling suppression.

Aims: The aims of this study are to investigate the pivotal role that raftlin molecular played in CD4⁺ T and B lymphocytes in ITP patients, and to provide new clues to the new treatment of ITP.

Methods: The raftlin level in the serum of ITP patients and normal controls was tested by ELISA. Then CD4⁺ T and B lymphocytes were isolated from the peripheral blood of ITP patients and normal controls. The isolated cells were incubated with anti-human raftlin antibodies and sent to detection by flow cytometry. IFN- γ and IL-17 expression were also measured with Th1/Th2/Th17 kits. Meanwhile, the raftlin mRNA expression was quantified in CD4⁺ T and B lymphocytes with Real-time PCR.

Results: Compared to normal controls, the raftlin level in the sera of ITP patients was significantly elevated. Both the percentage of B cells expressing raftlin and the MFI of raftlin in B cells were increased in ITP patients than normal controls. Quantitative analysis of raftlin mRNA expression in B cells was also higher in ITP patients. However, though the intracellular cytokines showed the increased level of IFN- γ and IL-17 in ITP patients, neither CD4⁺ T cells expressing raftlin nor the raftlin mRNA expression in CD4⁺ T cells of ITP patients exhibited significant difference compared with normal controls.

Conclusion: In ITP patients, raftlin was demonstrated to be associated with the B cells immune dysfunction.

Disclosure of Interest: None declared.

Inflammation I

PO321-MON

Neutrophil elastase is not required for neutrophil extracellular trap or deep vein thrombus formation in mice

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Background: The serine protease neutrophil elastase (NE) has been shown undergo nuclear translocation during neutrophil extracellular trap formation, resulting in histone degradation and thus aiding in chromatin decondensation. It has been proposed that NE-deficient (NE^{-/-}) mice are unable to decondense chromatin or release NETs in response to a microbial stimulus. However, mouse studies quantitatively evaluating the role of NE in NET formation remained to be performed.

Aims: We wished to establish if neutrophils to what degree NE^{-/-} mice would produce NETs during sterile inflammation and to examine whether this NE-deficiency impact venous thrombosis induced by stenosis, a model in which abundant NET release occurs in the absence of infection.

Methods: We analyzed NE^{-/-} neutrophils for NET formation in vitro and performed the inferior vena cava (IVC) stenosis model of deep vein thrombosis in wild-type (WT) and NE^{-/-} mice.

Results: We found that NE-deficiency only resulted in a slight reduction in NET formation in response to calcium ionophore and occurred normally in response to PMA. In addition, histone hypercitrullination was unaffected with either stimulus. NE^{-/-} mice formed thrombi of similar size and with similar frequency to WT mice after either 6 or 48 h of IVC stenosis. Diffuse, extracellular H3Cit was prominent in thrombi from mice of both genotypes.

Conclusion: Therefore, NE^{-/-} mice do not phenocopy PAD4^{-/-} mice that are greatly protected from thrombus formation and indicate that NE inhibition would not be a good target in DVT. Mouse experiments using NE inhibitors with the goal of inhibiting NET formation are therefore not recommended. Our study also highlights that NET-targeted therapies would need to be highly effective in order to have a substantial impact.

Disclosure of Interest: None declared.

PO322-MON

Tissue factor-dependent platelet activation supports neutrophil recruitment, rolling and transmigration during inflammation

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Background: Transendothelial migration of neutrophils in venules constitutes a key event in the inflammatory response. In the current view, neutrophil recruitment occurs through selectin- and integrin-dependent interactions with the inflamed endothelium. Additionally, platelets actively participate in inflammatory reactions and atherosclerosis by the action of proinflammatory chemokines.

Aims: In this present study we propose to determine the involvement of the blood coagulation cascade and platelets on inflammation, with a sterile inflammation mice model in real time using a multichannel intravital spinning disk confocal microscope.

Methods: We used a sterile inflammation mice model in real time using a multichannel intravital spinning disk confocal microscope.

Results: In the seconds following an inflammatory stimulus, neutrophils adhere to the inflamed endothelium through ICAM-1 and lymphocyte function-associated antigen-1 (LFA-1) interactions, become activated, and express TF, which induces platelet activation and fibrin formation. Activated platelets bound to vWF surround the inflamed vessel and express P-selectin. Circulating neutrophils roll on platelet P-selectin and adhere to platelets and fibrin clots before transmigration. Last, infusion of wild-type neutrophils into mice expressing low levels of human TF ($\approx 1\%$) and wild-type platelets into P-selectin null mice restored platelet adhesion and activation; fibrin formation; and the rolling, adhesion, and transmigration of neutrophils.

Conclusion: Taken together, our results indicate that platelets constitute a third cellular partner and together with the TF play a key role in the innate immune response. Our data identify a previously unknown role for the coagulation cascade and platelets in neutrophil recruitment and highlight the close relationship between the hemostatic system and inflammation.

Disclosure of Interest: None declared.

PO323-MON

Effect of glucose levels in increased neutrophil extracellular trap formation (NETs) in type 2 diabetes patients at diagnosis and after 6 and 12 months of metabolic control

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Background: Diabetes is characterized by a low grade chronic inflammatory state, endothelial dysfunction, an increased risk of infections and early cardiovascular disease. The release of NETs is a mechanism by which neutrophils in concerted action with platelets and endothelium, kill bacteria and exerts proinflammatory and prothrombotic activities.

Aims: To explore the association between blood glucose reduction and changes in NET formation and other inflammatory markers in recently diagnosed type 2 diabetic patients (RDT2DP).

Methods: RDT2DP ($N = 12$, glycemia 210 ± 29 mg dL⁻¹, HbA1c $9.6 \pm 0.5\%$) and healthy donors ($N = 12$) were included after obtaining informed written consent from all participants. NET formation was studied by fluorometry labelling cells with Sybr Gold. Nucleosomes and von Willebrand factor plasmatic levels (vWF) were measured by ELISA and platelet P-selectin by cytometry. All parameters were evaluated at diagnosis and after 6 and 12 months of metformin treatment.

Results: While unstimulated neutrophils from donors did not form NETs, RDT2DP exhibited spontaneous NET formation (0.2 ± 0.04 vs. 0.5 ± 0.1 mg mL⁻¹ of DNA, $P < 0.05$, Student t-test). Although TNF α stimulation of control neutrophils triggered DNA release (0.6 ± 0.1 mg mL⁻¹) patient's neutrophils were not responsive (0.5 ± 0.1 mg mL⁻¹). While glycemia and HbA1c levels were normalized after 6 months of treatment (117 ± 6.1 mg dL⁻¹ and 6.5 ± 0.2 , respectively), basal (0.3 ± 0.1 mg mL⁻¹) and TNF α -mediated NET formation reached normal values (0.6 ± 0.1 mg mL⁻¹) after 12 months. Nucleosome levels were higher in patients than in controls (0.6 ± 0.20 vs. 1 ± 0.02 , $P < 0.05$), and decreased after 12 months (0.3 ± 0.1 mg mL⁻¹). P-selectin expression (% of positive cells control: 6 ± 1 vs. patients 7 ± 2), and vWF levels (control: 5.7 ± 0.6 vs. patients: 4.7 ± 0.5 mg mL⁻¹) were similar in both populations.

Conclusion: Our preliminary data suggest that NETs could represent a novel inflammatory biomarker in type 2 diabetes and that increased nucleosome levels seems not to be a consequence of impaired glycemic control.

Disclosure of Interest: None declared.

PO324-MON

Impact of PCSK9 in a murine mouse model of sepsis

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Background: Proprotein convertase subtilisin/kexin type 9 (PCSK9), plays a key role in low density lipoprotein (LDL) degradation. Loss of function of PCSK9 exerts a protective effect in sepsis in both mice and humans, possibly via increased pathogen lipid clearance. In contrast, overexpression of PCSK9 and PCSK9 gain of function variants exhibit

increase in circulating LDL cholesterol and an increased risk of cardiovascular disease.

Aims: To study the role of PCSK9 in a murine model of polymicrobial sepsis, using cecal-ligation and puncture (CLP). We hypothesize that reduced function of PCSK9 has a protective effect in sepsis, while overexpression of PCSK9 in mice exacerbates the proinflammatory and procoagulant responses.

Methods: Wild-type (*Pcsk9*^{+/+}), PCSK9 KO (*Pcsk9*^{-/-}) and PCSK9 overexpressing (*Tg-Pcsk9*) male mice were subjected to intra-abdominal sepsis using CLP and Sham surgery (no ligation or puncture). Eight hours post-surgery, blood was collected via the inferior vena cava. Lung myeloperoxidase (MPO) activity, plasma levels of cell free DNA (cfDNA), Protein C (PC) antigen, thrombin-antithrombin (TAT) complexes, interleukin 6 (IL-6) and interleukin 10 (IL-10) were measured.

Results: All the mice subjected to CLP showed increases in levels of cfDNA, IL-6, IL-10, TAT complexes as well as Lung MPO activity, and decreased levels of circulating PC antigen as compared to sham operated mice. Interestingly, the *Pcsk9*^{-/-} mice subjected to CLP showed reduced levels of IL-6, IL-10, cfDNA, TAT complexes, PC and decreased Lung MPO activity in comparison to the CLP subjected WT mice. On the other hand, *Tg-Pcsk9* septic mice showed elevations in the levels of cfDNA, IL-6, IL-10, TAT complexes, PC and MPO activity as compared to the septic WT mice.

Conclusion: Our results showed that PCSK9 modulates inflammation and coagulation in septic mice. Thus, therapies that target PCSK9 might improve outcomes of sepsis.

Disclosure of Interest: None declared.

PO325-MON

Plasma cell-free DNA levels are elevated in patients with acute ischemic heart failure partially due to neutrophil extracellular trap formation

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Background: Heart failure (HF) is a serious clinical problem. Inflammation is an integral part of the HF pathogenesis. Inflammation is accompanied by the release of nuclear content resulting either from cell destruction or Neutrophil Extracellular Trap (NETs) formation. The presence and source of plasma DNA in HF patients remains unknown.

Aims: To measure levels of cell-free DNA in plasma in acute HF (AHF), correlate them with stable HF (SHF), non-HF and healthy individuals and with other indices of thromboinflammation as well as to verify to what extent this DNA originates from NETs.

Methods: Patients with ischemic AHF ($n = 50$) were compared to patients with SHF ($n = 38$), stable coronary artery disease (CAD) with no HF ($n = 38$) and healthy controls ($n = 35$). Plasma DNA levels were measured using SYTOX[®] Green and Synergy HT fluorescence microplate reader.

Results: Individuals with AHF had elevated plasma DNA levels compared to all other study groups ($P < 0.001$). In patients with AHF, amount of plasma DNA directly correlated with neutrophil count ($r = 0.38$, $P = 0.035$) and levels of high sensitivity C-reactive protein ($r = 0.43$, $P = 0.002$) and interleukin-6 ($r = 0.44$, $P = 0.002$). Correlation with lymphocyte count was inverse ($r = -0.49$, $P = 0.004$). An insignificant trend to decrease in DNA levels was observed during 3-month follow up period ($P = 0.13$). DNA levels (assessed as quartiles using the Cox regression analysis) were insignificantly predictive of the end-point "death or re-hospitalization" or death alone. Western blot

showed citrullinated histone 3 (CitH3, a hallmark of NETs) in 4 AHF ($n = 11$) and 1 SHF ($n = 4$) plasma samples containing the highest levels of DNA. No CitH3 was observed in healthy controls or CAD ($n = 4$ and 3, respectively).

Conclusion: Free plasma DNA levels are increased in AHF where they correlate with neutrophil count and plasma inflammation markers and inversely correlate with lymphocyte count. NETs may account for the elevated DNA amounts in a part but not all patients.

Disclosure of Interest: None declared.

PO326-MON

The histone-cleaving factor seven activating protease does not promote degradation of neutrophil extracellular traps

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Background: Increased Factor VII activating protease (FSAP) antigen and activation have been linked to sepsis, carotid and coronary artery stenosis. FSAP circulates as an inactive zymogen, and is (auto-) activated upon binding to charged molecules including histones, DNA and RNA, and upon contact with apoptotic or necrotic cells. We have shown that FSAP greatly accelerates the release of nucleosomes from late apoptotic and, in the presence of DNase, also necrotic cells, and that histone H1 is cleaved in this process. Recently, it has been shown that histones and nucleosomes are released in the form of neutrophil extracellular traps (NETs) in sepsis, experimental stroke, and several thrombotic and inflammatory diseases. NETs/histones are highly cytotoxic and prothrombotic and we hypothesized that FSAP activation in these diseases may aid NETs clearance through histone proteolysis.

Aims: To determine whether FSAP is activated upon contact with NETs and promotes the degradation of NETs in plasma/serum.

Methods: Neutrophils were stimulated with PMA to induce NETs and incubated with FSAP containing serum or recombinant FSAP. FSAP binding to NETs was studied by immunofluorescence microscopy. FSAP- $\alpha 2$ -antiplasmin (AP) complexes were measured as indirect marker for FSAP activation. NETs degradation was determined using Picogreen assay for released DNA, and nucleosome ELISA.

Results: Using confocal microscopy we found that FSAP appears to colocalise with NETs. Moreover we found that nucleosomes in NETs were degraded upon incubation with pre-activated recombinant FSAP. However, there was no difference between NETs DNA degradation in the presence of normal or FSAP deficient serum, and FSAP even appeared to protect NETs DNA from degradation by added DNaseI. Surprisingly, no FSAP activation by NETs was detected by measuring FSAP complex formation with the plasma serpin AP.

Conclusion: Our results suggest that whilst FSAP binds to NETs this does not lead to its activation and the degradation of NETs.

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PO327-MON

Mechanism of ϵ -viniferin inhibits superoxide generation in human neutrophil

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Background: Neutrophils is the most abundant immune cells in human body. The undesirable respiratory burst in neutrophils leads to pathological inflammation and tissue damage. Neutrophils play a important role in our innate immune system.

Aims: This study investigates the effect and the underlying mechanism of ϵ -Viniferin (VT-1), a lignan extracted from the *Vitis thunbergii* Sieb. & Zucc. var. *thunbergii*, on *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP)-induced respiratory burst in human neutrophils.

Methods: Signaling pathways regulated by ϵ -Viniferin to oppose fMLP-induced respiratory burst and cathepsin G release were evaluated by receptor binding assay, other agonist-induced respiratory burst, phosphorylation of other proteins induced by fMLP and other agonists.

Results: ϵ -Viniferin inhibited fMLP-induced superoxide anion production ($IC_{50} = 2.30 \pm 0.96 \mu M$, mean $\pm$ SEM, $n = 6$), cathepsin G release ($IC_{50} = 30.15 \pm 2.59 \mu M$, $n = 3$) in a concentration dependent manner. Further, ϵ -Viniferin specific suppresses fMLP and receptor binding function ($IC_{50} = 4.36 \pm 1.74 \mu M$, mean $\pm$ SEM, $n = 6$). Consequently, ϵ -Viniferin attenuated ERK1/2 and Akt phosphorylation which are the downstream proteins of fMLP signaling. In addition, combine ϵ -Viniferin and the FPRL1 antagonist WRW4 can have additional effect in the superoxide anion production and fMLP and its receptor binding function.

Conclusion: ϵ -Viniferin specific opposes fMLP-mediated neutrophil respiratory burst by inhibiting fMLP bind with it receptors, specifically effect on the FPR. ϵ -Viniferin maybe is a FPR antagonist.

Disclosure of Interest: None declared.

PO328-MON

Inhibitory effect of NKSE-5a on superoxide anion generation in human neutrophils

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Background: Neutrophils play an important antimicrobial role in protecting the host from a range of infectious agents by the generation of reactive oxygen species (ROS). However, inappropriate ROS production damage host tissues, amplifying the response in inflammatory diseases.

Aims: The undesirable respiratory burst in neutrophils leads to pathological inflammation and tissue damage. This study investigates the effect and the underlying mechanism of 9-9'-O-diferuloyls-ecoisolariciresinol(NKSE-5A), a lignan extracted from the *Microtropis japonica*, on *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) and phorbol 12-myristate 13-acetate (PMA)-induced respiratory burst in human neutrophils.

Methods: Signaling pathways regulated by NKSE-5A to oppose fMLP and PMA-induced respiratory burst were evaluated by phosphorylation of Akt kinase induced by fMLP, Akt kinases activity and by immunoblotting analysis of the downstream targets of Akt kinase.

Results: NKSE-5A inhibited fMLP-induced superoxide anion production ($IC_{50} = 3.57 \pm 1.26 \mu M$, mean $\pm$ SEM, $n = 6$) in a concentration dependent manner. However, NKSE-5A did not inhibit superoxide anion production caused by PMA, cathepsin G release and calcium release neither caused by fMLP and PMA. Further, NKSE-5A non-

specific minor suppresses fMLP and PMA-induced Akt and Erk(p42/p44) phosphorylation. These results indicate that NKSE-5A inhibits fMLP and PMA induced superoxide production upstream of the NADPH oxidase via a mechanism involving Erk and Akt kinase phosphorylation.

Conclusion: NKSE-5A inhibits fMLP and PMA induced superoxide production upstream of the NADPH oxidase via a mechanism involving Erk and Akt kinase phosphorylation. P38, p47^{phox}, p40^{phox} etc, we have to confirm that NKSE-5A suppresses fMLP or PMA induced these protein phosphorylation or translocation to inhibit superoxide anion production.

Disclosure of Interest: None declared.

PO329-MON

Diabetes primes neutrophils to undergo netosis which severely impairs wound healing

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Background: Diabetes impairs wound repair resulting in significant morbidity and mortality. Neutrophils, being involved in the early phase of healing, can trap and destroy bacteria by extruding their DNA and cellular contents to form neutrophil extracellular traps (NETs). However, NETs are injurious to tissue and thus could delay healing. While NETs are recognized to fuel inflammation and thrombosis, how diabetes modulates NET formation (NETosis) and whether NETs impair diabetic wound healing remain unknown.

Aims: To test the hypotheses that NETs are overproduced in diabetes and that the impaired wound healing in diabetes is due to excessive NETs in wounds.

Methods: Neutrophils isolated from diabetic patients (type 1 and type 2) and mice (streptozotocin-induced diabetic mice and db/db mice) were subjected to in vitro NETosis assays. Protein expression of peptidylarginine deiminase 4 (PAD4), an enzyme important in chromatin decondensation preceding NETosis, was examined by Western blotting. Normoglycemic and diabetic wild-type (WT) and PAD4^{-/-} mice undergone excisional skin wounding were monitored for healing macro- and microscopically. DNase 1 was administered to evaluate its impact on wound healing.

Results: Neutrophils from both type 1 and type 2 diabetic patients and diabetic mice were primed for NETosis compared to normoglycemic controls. PAD4 was 4-fold elevated in neutrophils of diabetic patients. Higher levels of NET biomarkers were found in wounds of diabetic WT mice which suffered a significant delay in healing. Impressively, PAD4^{-/-} mice, which cannot make NETs, healed faster than WT and showed no delay in healing when made diabetic. DNase 1 accelerated wound healing in diabetic WT mice.

Conclusion: Our findings suggest that diabetes predisposes neutrophils to NET overproduction, which is a key factor delaying diabetic wound repair. PAD4 inhibition or NET cleavage may be a new therapeutic strategy to improve wound healing and reduce NET-driven thrombotic and inflammatory complications in diabetics.

Disclosure of Interest: None declared.

PO330-MON

Determinants of the regulatory action of platelets towards neutrophil activities

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Background: Platelets intervene at various stages of innate and adaptive immune responses. In various models of inflammation, platelets have been shown to promote leukocyte recruitment and infiltration, to modulate leukocyte activities, and to repair vascular damage associated with leukocyte trafficking. Platelets can either stimulate or inhibit neutrophil activities depending on the pathophysiological situation or experimental model considered, suggesting that regulation of neutrophil functions is dependent on the inflammatory context. However, the factors that determine the pro- or anti-neutrophil activity of platelets remain to be determined.

Aims: To identify the determinants of the regulatory action of platelets towards neutrophil secretion and oxidative activity.

Methods: Isolated human neutrophils were co-incubated with washed platelets and stimulated or not with tumor necrosis factor alpha (TNF- α). In some experiments cell culture inserts were used to prevent the contact between the two cell types. Platelet agonists and inhibitors were also used to modulate the state of activation of platelets. For each experimental condition, the activity and secretion of elastase and myeloperoxidase (MPO), as well as the production of reactive oxygen species (ROS) by neutrophils were quantified.

Results: While platelets had no effect on the activity of purified elastase or MPO, they reduced significantly the secretion of these two enzymes as well as ROS production in both unstimulated and TNF- α -stimulated neutrophil suspensions. These inhibitory effects of platelets were enhanced by thrombin and fibrillar collagen. In contrast, when the contact between platelets and neutrophils was prevented, platelets lost their ability to downregulate neutrophil activities.

Conclusion: Our results indicate that regulation of neutrophil injurious activities by platelets relies on direct interaction between the two cell types and on the state of activation of platelets.

Disclosure of Interest: None declared.

PO331-MON

The leukocyte integrin $\beta 2$ primes neutrophils for netosis in sickle cell disease

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Background: Approximately 100 000 people are affected by Sickle Cell Disease (SCD) in the USA; moreover, the disease is considered a global public health problem. SCD is a chronic inflammatory disease marked by more activated neutrophils. Neutrophils can extrude their decondensed chromatin to form structures called neutrophil extracellular traps (NETs) that kill circulating pathogens. Although NETs are often beneficial to the host, NETs can lead to lethal complications in several diseases.

Aims: Our aims are to determine the extent to which NETs are involved in SCD pathophysiology and to investigate the potential mechanisms leading to NETosis (death by NETs).

Methods: NETosis was assessed in isolated neutrophils from consented SCD patients and non-SCD donors, treated with NETosis agonists and stained with a DNA dye. NETosis was assessed by quantifying the distinctive decondensed nuclei.

Results: Neutrophils isolated from SCD patients and stimulated with phorbol 12-myristate 13-acetate (PMA) or glucose oxidase exhibited

more NETosis than non-SCD donors. Treatment with integrin $\beta 2$ and αM but not $\beta 1$, αL or αX function blocking antibodies significantly attenuated NETosis, suggesting the involvement of $\alpha M\beta 2$ in SCD-related NETosis. In addition, neutrophils from integrin $\beta 2$ knockout mice had reduced NETosis. Furthermore, a higher number of SCD neutrophils expressed the activated form of integrin $\beta 2$ (78% SCD vs 53% controls) and αM (55% SCD vs 16% control). Unstimulated SCD neutrophils expressed higher levels of pERK1/2, which is a necessary signaling kinase in NETosis.

Conclusion: Our study is the first to indicate that neutrophils of human SCD patients are more sensitive to NETosis in part, due to activation of integrin $\alpha M\beta 2$. Therefore, targeting this specific integrin has potential in attenuating NET-associated complications in SCD.

Disclosure of Interest: None declared.

PO332-MON

Neutrophil incorporation of membrane fragments from dying platelets

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Background: Activation of the apoptotic or necrotic death pathways in platelets can induce phosphatidylserine (PS) expression. We have recently demonstrated that necrotic platelets produce high levels of the proinflammatory lipid PAF and are highly reactive to leukocytes, raising the possibility that these platelets may have proinflammatory functions.

Aims: To investigate the biophysical interactions between necrotic PS⁺ platelets and neutrophils under varying hemodynamic shear stress.

Methods: Neutrophils were perfused over PS⁺ and PS⁻ platelet monolayers or thrombi, and PS⁺ platelet-neutrophil interactions monitored.

Results: Our studies have revealed that rolling neutrophils extract membrane fragments from the surface of necrotic PS⁺ platelets at physiologic shear rates. The stability of platelet membranes was determined by exposing PS⁺ platelet monolayers to sequential increases in shear. Membrane deformation and fragmentation was observed at 1,800s⁻¹ (corresponding to a drag force of 4.5pN mm⁻¹), progressing to full platelet detachment at 28 800 s⁻¹ (72pN mm⁻¹). Estimation of the drag forces imposed by rolling neutrophils on PS⁺ platelets revealed a force of 3.85pN mm at 150s⁻¹ when neutrophil adhesion was distributed evenly over the entire spread platelet (10 mm), increasing up to 385pN mm⁻¹ when neutrophils pulled localized membrane fragments (0.1 mm). The PS⁺ platelet instability correlated with the progressive loss of filamentous actin, a process greatly lowering the neutrophil drag force required for membrane fragmentation. Notably, numerous proinflammatory molecules expressed on PS⁺ platelet surfaces became associated with the rolling neutrophils.

Conclusion: PS⁺ platelets have fragile membranes that fragment under the influence of hemodynamic drag forces imposed by rolling neutrophils. This fragility may be important to promote leukocyte inflammatory function by presenting surface expressed and granule stored inflammatory molecules to rolling leukocytes.

Disclosure of Interest: None declared.

Microparticles I

PO333-MON

General decrease of circulating microparticles is related to the presence of FVIII inhibitors in hemophilia A patients

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Background: The cell activation in the blood is associated with plasma membrane remodeling, resulting in the shedding of membrane-derived microparticles (MPs) rich in accessible phosphatidylserine and in specific cell-surface antigens. There are limited data about the role of MPs in hemophilia A [HA] and especially in FVIII inhibitors development.

Aims: This study aimed to assess the MPs dosage in patients with HA and positive [HA α -FVIII(+)] ($n = 34$) or negative [HA α -FVIII(-)] ($n = 68$) for inhibitors.

Methods: The study was conducted in Fundação Hemominas, Belo Horizonte, MG, Brazil. The recruitment of participants and the methodology of the study were approved by the Brazilian ethics committees. MPs were analyzed by flow cytometry using the classical MPs marker, annexin, and fluorochrome-labeled monoclonal antibodies against specific cell surface markers: T cells (CD3); platelets (CD41a); granulocytes (CD66); leukocytes (CD45); erythrocytes (CD235a); and endothelial cells (CD51/61).

Results: The meaning of circulating MPs were markedly increased in HA α -FVIII(-) when compared to HA α -FVIII(+) in T cells (33.58 ± 15.08 vs 13.20 ± 14.70 ; $P < 0.01$); platelets (203.10 ± 450.00 vs 50.86 ± 54.64 ; $P = 0.03$); granulocytes (12.05 ± 7.95 vs 6.78 ± 6.08 ; $P < 0.01$); leukocytes (241.10 ± 419.30 vs 71.29 ± 45.87 ; $P = 0.02$); endothelial cells (31.57 ± 11.42 vs 22.22 ± 11.41 ; $P < 0.01$) (MPs/ μ L $\pm$ standard deviation). In contrast, the meaning MPs μ L⁻¹ of erythrocytes were higher in HA α -FVIII(+) (12.64 ± 10.01 vs 8.58 ± 9.53 ; $P < 0.01$).

Conclusion: We found recently that HA α -FVIII(-) patients has increased activation of all leukocytes and synthesis of pro-inflammatory cytokines. These data could explain the increased presence of MPs in these patients. Increases in circulating MPs counts may be a good prognostic for inhibitors clearance and could be used as an important biomarker for the inhibitors development. More research is needed to fully understand the role of MPs in hemophilia A. Financial support: Fundação Hemominas, Fapemig, CAPES and CNPq.

Disclosure of Interest: None declared.

PO334-MON

Pro-coagulant actions of circulating microparticles in sickle cell anemia and sepsis associated coagulopathy and their modulation by a triblock polymer MST 1-88

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Background: Circulating microparticles (MPs) represent vesicles and cellular fragments which are generated in both sickle cell anemia (SCA) and sepsis associated coagulopathy (SAC). Veploxamer is a triblock polymer with affinity to hydrophobic surfaces which are generated in microangiopathic conditions.

Aims: To test the hypothesis that Veploxamer may decrease the pro-coagulant effects of MPs, a functional method to access MPs levels and a thrombin generation assay was used.

Methods: Citrated and EDTA whole blood samples ($N = 25$) were collected from normal individuals, SCA patients ($n = 10$) and confirmed SAC patients ($n = 10$). Individual aliquots of these samples were supplemented with saline and Veploxamer at a range of 0.1–10 mg mL⁻¹. After 30mins of incubation these mixtures were centrifuged at 2500 g and plasma was retrieved. These samples were analyzed for functional levels using an annexin binding amidolytic method for MPs mediated thrombin generation, and TF mediated thrombin generation using a flurometric method.

Results: In comparison to normal (18.4 ± 5.1 nm), the MPs levels were significantly higher in the SCA (35.1 ± 9.4 nm) and SAC patients (26.1 ± 8.2 nm). The functional level of MPs was markedly reduced in the Veploxamer supplemented normal (8.3 ± 1.4 nm), SCA (16.1 ± 4.6 nm) and SAC (12.6 ± 2.8 nm). In the thrombin generation assays in contrast to normal (240 ± 28 nm) SCA patients (320 ± 48 nM) and in SAC patient's plasma (349 ± 68 nm) generated higher levels of thrombin. Supplementation of Veploxamer resulted in significant decrease in thrombin generation in normal (108 ± 12 nm), SCA patient's plasma (1480 ± 181 nm) and SAC patient's (158 ± 22 nm) groups.

Conclusion: Both SCA and SAC are associated with vasculopathy due to the deposition of fibrin in the microvasculature resulting in the generation of MPs from cellular deformation and damage. Supplementation of Veploxamer to both groups of blood samples resulted in a marked decrease of functional MPs and thrombin generation.

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PO335-MON

The role of microvesicles derived from myeloma cells in thrombogenicity and angiogenesis

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Background: Multiple myeloma (MM) is a malignant disease characterized by increased angiogenic activity and thrombogenicity. The properties of myeloma cell derived microvesicles (MM-MVs), potentially serving as vehicles for cytokines and coagulation factors, are not fully elucidated.

Aims: The current study was aimed to characterize MM-derived MVs, focusing on their role in angiogenesis and thrombogenicity and explore the impact of proteasome inhibitor therapy [Bortezomib (Bort)] on these biological processes and MV profile.

Methods: MVs were generated from MM cell line RPMI 8226 untreated or treated with 100 nM Bort. Properties of MM-MVs were tested (FACS, protein arrays) and their coagulation activity was checked by the FXa chromogenic assay. The migratory capacity (migration assay), proliferation rate (XTT assay) and cell signaling effects (Western blot analysis) of MVs on endothelial cells (ECs) were analyzed.

Results: MM-MVs expressed MM-derived markers (syndecan-1/CD138, CD38) as well as coagulation (TF, TFPI, EPCR, TM) and angiogenic-related factors (VEGFR1, VEGFR2, and CD31). They also contained angiogenic antigens (angiogenin, PDGF-BB and VEGF) and displayed procoagulant activity. Both untreated and Bort-treated MM-MVs penetrated ECs. However, while MVs obtained

from untreated cells increased migration and proliferation capacity of ECs (by 13 and 1.4 folds respectively), MVs derived from cells treated with Bort failed to induce these effects. MVs of untreated cells increased ERK1/2 and c-Jun phosphorylation in ECs (by 6.15 and 1.84 folds) but did not affect MAPKAPK-2. In contrast, MVs of Bort treated cells reduced c-Jun phosphorylation (by 27%) in ECs.

Conclusion: The expression of angiogenic and coagulation factors on MM-MVs and their penetration to ECs may play a role in EC proliferation and migration, resulting in increased angiogenesis. MVs of Bort treated cells suppress these effects, thereby potentially contributing to MM regression.

Disclosure of Interest: None declared.

PO336-MON

Antithrombin is incorporated into exosomes and its presence in these vesicles is modulated by low molecular weight heparin

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Background: Exosomes are 40–100 nm vesicles of endocytic origin which are produced by normal and tumor cells. They mediate cell-cell communication regulating processes such as inflammation and metastasis. They content proteins and different species of RNA. By proteomic analysis of exosomes from MDCK cells (canine kidney epithelial cells) we found that bovine antithrombin (AT) was present. AT is the main inhibitor of the coagulation. However, it has been shown that AT exerts other roles beyond hemostasis, such as anti-inflammatory, anti-angiogenic, anti-apoptotic and anti-viral. Heparin is the main co-factor of AT and it binds to a positively charged domain of AT called heparin binding site (HBS) inducing an activating conformational change. However, the anti-inflammatory and anti-angiogenic roles of AT depend on its binding to heparan sulfate proteoglycans (HSPG) through this HBS, which is blocked in presence of heparin.

Aims: To study if exogenous AT binds to cell membrane receptors that are later incorporated into exosomes and whether heparin can modulate this process.

Methods: Isolation and purification of exosomes from MDCK cells, SDS-PAGE and Western blot, trypsin treatment of exosomes and Electron Microscopy.

Results: Levels of AT in exosomes were higher in those obtained from cells incubated in presence of low molecular weight heparin (LMWH), and it was no present in cell lysates and released microvesicles. This result suggests that LMWH favors the interaction of AT to its receptor, which is unlikely an HSPG. Moreover, a fraction of AT of exosomes had a higher molecular weight in SDS-PAGE under reducing conditions, which suggests a covalent complex with another protein. Treatment of exosomes with 0.05% trypsin, degraded AT without affecting the exosome integrity, suggesting that AT is on the exosome surface.

Conclusion: Exogenous AT is incorporated into the surface of MDCK-exosomes but its role and how this role is modulated by LMWH is currently under investigation.

Disclosure of Interest: None declared.

PO337-MON

Single case report: monitoring the balance of microparticle-associated procoagulant and fibrinolytic potentials after orthopedic surgery

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Background: Cell-derived microparticles (MPs) vehiculate either procoagulant or pro-fibrinolytic potentials via phosphatidylserine (PS) +/- tissue factor (TF) expression or via the uPA-uPA_r complex respectively (1). These functions can now be measured on purified MPs following plasma extraction, but their impact on hemostasis is still unclear.

Aims: This single case observational study aimed to monitor potential modulations of both MP potentials in the course of orthopedic surgery.

Methods: One staff member was submitted to knee surgery and regularly donated blood samples, everyday around surgery and on a weekly basis during standard LMWH treatment. Platelet-free plasma (PFP) was generated from citrated blood by sequential centrifugations (2500 g 15 min twice) and aliquoted for -80 °C storage. TF-dependent procoagulant activity was measured in a FXa generation assay on MPs purified by centrifugation (24 000 g, 1 h), as modified from (2) with femtomolar sensitivity. MP-linked plasmin generation capacity (MP-PGC), characteristic of fibrinolytic potential, was measured on myeloid leucocyte-derived MPs isolated from plasma by CD15-coated magnetic beads with a prototype assay using a plasmin-specific chromogenic substrate.

Results: As also observed in healthy donors, baseline levels of MP-TF activity were low albeit not null (10 ± 3 fM TF). Slightly higher (1.5 to 2x) than baseline and pre-op. levels were measured immediately post-op, 24 h and 48 h after intervention. Compared to baseline MP-PGC, high and sustained levels (4–6x normal, d + 13 to d + 40) were observed when LMWH dosage was increased (4.5 to 18 000 IU per day) to fight against leg micro-phlebitis.

Conclusion: These limited observations suggest that MP fibrinolytic potential may be elevated just after recent thrombus formation and/or as a consequence of enhancing heparin treatment, thus favoring plasmin generation and clot dissolution via uPA-uPA_r MPs. They advocate for future studies in orthopedic surgery. 1) Lacroix R, JTH 2013. 2) Lee RD, Thr Res 2012.

Disclosure of Interest: P. Poncelet Employee of: BioCytex, C. Judicone Employee of: BioCytex, J. Bez Employee of: BioCytex, T. Bouriche Employee of: BioCytex, B. François: None declared, E. Forestier: None declared, O. Béréziat: None declared, J. Sampol: None declared, R. Lacroix: None declared, F. George: None declared.

PO338-MON

Patients with unprovoked venous thromboembolism have higher concentration of large extracellular vesicles with augmented potential to initiate coagulation activation

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Background: Extracellular vesicles (EVs) are small membrane vesicles (50–1000 nm) released from activated cells that may play an important role in the pathogenesis of thrombotic diseases.

Aims: To investigate whether patients with unprovoked venous thromboembolism (VTE) have different concentration, size distribution, and pro-coagulant potential of plasma EVs than age- and sex-matched healthy controls.

Methods: A case-control study was performed in 23 patients with a history of incident unprovoked VTE 1–5 years prior of the study, and 23 age- and sex-matched healthy controls recruited from the general population. The plasma concentration and size distribution of EVs was measured using NanoSight NS300. The pro-coagulant potential of EVs was tested by the calibrated automated thrombogram (CAT) assay. Purified EVs from patients and controls was added without and with 3.0 pM tissue factor (TF) to MP-free plasma (MPFP) prepared by ultra-centrifugation (100 000 g, 1 h). The study was approved by the research ethics committee and all subjects gave informed written consent.

Results: At baseline we observed an association between levels of EVs and the risk of VTE (1 standard deviation increase of respectively total EV count [odds ratio 0.9, 95% confidence interval 0.5–1.6], <100 nm [OR1.2, 95%CI 0.6–24], 100–199 nm [OR1.4, 95%CI 0.6–3.1], 200–299 nm [OR1.5, 95%CI 0.5–4.4], 300–1000 nm [OR1.7, 95%CI 0.4–6.4]). Purified EVs from VTE patients induced shorter lag-time than EVs from healthy controls (3.4 ± 1.9 vs. 4.1 ± 2.2 min, $P < 0.01$) (mean $\pm$ 1SD), whereas the endogenous thrombin was similar for VTE patients (2140 ± 185 nM*min) and controls (1980 ± 147 nM*min, $P = 0.18$).

Conclusion: Our findings imply that VTE patients have higher plasma concentrations of large EVs with augmented ability to initiate coagulation activation compared to healthy controls. Risk of VTE was 1.7-fold increased for 1 SD increase in EVs of 300–1000 nm of size.

Disclosure of Interest: None declared.

PO339-MON

Influence of CALR and JAK2 mutations on procoagulant potential of platelet-derived microparticles in essential thrombocythemia (ET)

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Background: ET is a myeloproliferative neoplasm characterized by high thrombotic risk and JAK2V617F and CALR mutations. In plasma of ET patients, we recently found increased levels of circulating microparticles (MP) and MP-associated procoagulant activity (PCA).

Aims: To evaluate: 1. whether the elevated plasma MP-associated PCA is due to an increased production of platelet-MP (PMP) and/or an increased procoagulant potential of PMP; and 2. whether these properties are influenced by CALR or JAK2V617F mutations.

Methods: We studied 25 ET and 20 control subjects (CTR). Flow cytometry (Accuri C6, BD) was used to quantify MP in plasma and MP released in vitro by platelets. PMP thrombin generation (TG) potential was measured by calibrated automated thrombography (Stago), and PMP dependent PCA by P-PPL/1 assay (Stago).

Results: The *in vivo* study showed that plasma PMP concentration was significantly ($P < 0.05$) higher in ET patients compared to CTR; however, when PMP counts were expressed as a percentage of the platelet count, the PMP/platelet ratio was significantly ($P < 0.05$) lower in ET versus CTR. No statistically significant differences were observed between CALR-pos and CALR-neg/JAK2-neg and JAK2-pos patients. The *in vitro* study, in unstimulated platelets, showed that ET platelets released a significantly lower number of PMP compared to CTR. Analysis according to mutations showed among JAK2-neg patients no significant differences between CALR-pos and CALR-neg subjects, while JAK2-pos patients released in vitro more PMP compared to CALR-pos subjects. ET-PMP expressed lower TG and PCA potential compared to CTR ($P < 0.05$). Of interest, PMP from JAK2-pos patients had the highest procoagulant potential.

Conclusion: Our results demonstrate that the observed higher PCA of MP *in vivo* is mainly due to the presence of elevated MP count in plasma and not to an increased MP intrinsic procoagulant activity. Furthermore, the difference in the type of genetic mutation influences the release of PMP and their procoagulant potential.

Disclosure of Interest: None declared.

PO340-MON

TF- and UPA-bearing microparticles modulate fibrin nanostructure

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Background: Hemostasis reactions require membrane surfaces provided by activated cells and cell-derived microparticles (MPs). MPs can vehiculate either pro-coagulant via PS +/- tissue factor (TF) or pro-fibrinolytic potential via the urokinase (uPA-uPAR) complex or both on the same MP subsets (1). These functions can be measured on MPs, but their impact on fibrin structure is still unclear.

Aims: We investigated how the different MPs modulate fibrin structure formation, stability and lysis using our recent real-time measurement of fibrin nanostructure (2).

Methods: *In vitro* models were used to generate MPs: TF-MPs from LPS-activated blood, plasmin-generating MPs from a whole blood aging model, PS+ platelet-derived MPs (PMP) from ionophore-activated platelets and cancer-derived MPs with both functions from culture supernatants of two cancer cell lines. MPs were characterized by Flow Cytometry and in house FXa- or Plasmin-generation assays on extracted MPs. MP-free plasma (MPFP) was spiked with dilutions of MPs bringing various levels of PS, TF- and/or uPA- activities, incubated with low TF plus t-PA and triggered by calcium. The number of protofibrils (Np) was measured in real time during 30 min at 37 °C on a prototype routine instrument (2, 3).

Results: TF-MPs increased fibrin formation rate. Both TF-MPs and PMP increased fibrin stability and plateau duration, whereas uPA-MPs increased clot lysis rate. Tumor-MPs showed both antagonistic effects depending on TF and/or uPA activities, with procoagulant effect and Np decrease and/or accelerated lysis. PMP, TF- and uPA-MPs modulate fibrin nanostructure.

Conclusion: These experiments suggest that a disruption of the balance between coagulation and fibrinolysis by the PMP, TF- and uPA-MPs may contribute to enhance hemorrhagic or thrombotic risk. MP lytic activity on fibrin clots could be a potential new determinant of thrombotic risk, mainly in cancer. (1) Lacroix et al. JTH 2013 (2) Dassi et al. ISTH 2015 (submitted), (3) Yeromonahos et al. Biophysical J. 2010, ATVB 2012

Disclosure of Interest: None declared.

PO341-MON

Modulating thrombotic diathesis in hereditary thrombophilia: a role for circulating microparticles?

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Background: Hereditary thrombophilia increases the risk of venous thromboembolism (VTE) through the impairment of natural anticoagulant pathways or the potentiation of procoagulants. Even in severe thrombophilia additional triggering factors are required to cause VTE.

Aims: To study source and procoagulant activity of microparticles (MP) in carriers of mild (factor V Leiden (FVL) and prothrombin gene mutation (PTM)) and severe (antithrombin (AT), protein C (PC) and protein S (PS) deficiency) hereditary thrombophilia.

Methods: Plasma level of annexin V-MP (AMP), endothelial- (EMP), platelet-derived (PMP), tissue factor-bearing (TF+) and MP procoagulant activity (PPL) was measured in 142 carriers of FVL, 124 of PTM and in 132 carriers of natural anticoagulant deficiencies (NAD) (25 AT, 63 PC and 64 PS defect). Age and gender-matched healthy individuals acted as controls.

Results: Each group of thrombophilic carriers showed higher median levels of AMP, EMP, PMP, TF+ and PPL activity than controls ($P < 0.001$, <0.001 , <0.01 , 0.025 and 0.03 , respectively). In the FVL and PTM group, homozygous subjects presented with higher MP levels than heterozygous subjects. Carriers of FVL with high levels of AMP and TF+ had an adjusted OR for VTE of 3.08 (95% CI, 1.42 - 6.69) and 2.28 (95% CI, 1.07 - 4.85), respectively. Carriers of PTM had an adjusted OR for VTE of 1.95 (95% CI, 1.0 - 4.33) for high AMP and 5.72 (95% CI, 1.96 - 6.67) for PMP. NAD carriers with high levels of AMP, EMP and PMP had an adjusted VTE OR of 3.36 (95% CI, 1.59 - 7.11), 9.26 (95% CI, 3.55-24.1) and 2.72 (95%CI, 1.16-6.38) respectively.

Conclusion: In both mild and severe thrombophilia circulating MP appear to contribute to VTE development. MP may be acting as the triggering factors to enhance the global prothrombotic state. The actual triggering MP type may vary according to thrombophilia - highest OR for VTE were: TF+ in FVL, PMP in PTM and EMP in the NAD. Further studies are needed to better define this association and its clinical relevance.

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PO342-MON

Circulating extracellular vesicle (EV) after acute hindlimb ischemia

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Background: Extracellular vesicles (EVs) play important roles in cardiovascular disease. Regarding vascular ischemia, there is increasing

evidence of direct involvement of EV in a variety of processes such as inflammation and neovascularization. Therefore, the analysis of EV is thought to provide novel information regarding the underlying cellular mechanisms of ischemia.

Aims: To determine changes in the numbers and cellular origin of EVs after hindlimb ischemia in WT mice.

Methods: Platelet poor plasma was obtained at baseline and 3, 15 and 28 days after hindlimb ischemia. The cellular origin of platelet-, (activated) endothelial- and neutrophil-derived EVs was measured with Apogee A50-Micro flow cytometer using antibodies against mouse CD41, CD62E, Ly6G, respectively. Comparisons between groups were performed with one-way ANOVA followed by Dunnett's multiple comparisons test.

Results: Platelet EVs tend to increase after ischemia (from $1.9 \times 10^7 \pm 1.7 \times 10^6$ vesicles/mL at baseline to $6.6 \times 10^7 \pm 1.9 \times 10^7$ vesicles/mL at day 28, P for trend <0.01). Endothelial EVs were lower 3 and 28 days post-ischemia compared to baseline ($P < 0.05$), and significantly increased at day 15 ($4.1 \times 10^7 \pm 2.3 \times 10^6$ vesicles/mL at baseline vs. $5.8 \times 10^7 \pm 5.5 \times 10^6$ vesicles mL⁻¹ at day 15, $P < 0.05$). Neutrophil EVs were below the detection limit in all samples.

Conclusion: Lower limb ischemia induces clear changes in the concentrations of EVs from different cell types during the regeneration process. We hypothesize that the analysis of these vesicles may be useful to obtain information regarding the cellular activation state during muscle regeneration in an animal model of hindlimb ischemia.

Disclosure of Interest: None declared.

PO343-MON

Comparison of intracardiac and venous blood levels of microvesicles, tissue factor and procoagulant phospholipids in patients with and without atrial fibrillation

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Background: Stroke is a common complication of atrial fibrillation (AF), and AF is the most common cause of stroke. Anticoagulant therapy reduces the risk of stroke considerably, but the underlying cause of this procoagulant state is not clear. Thrombus material is mainly formed in the left atrial appendage (LAA), where the endothelium may be deteriorated. In recent years microvesicles (MV) containing tissue factor (TF) and procoagulant phospholipids (PPL) have been shown to be thrombogenic, and MV may arise as a result of cell activation or apoptosis. We hypothesized that a cause of hypercoagulation in AF may be formation of MV in the LAA.

Aims: The aim of this study was to compare the amount of MV, TF and PPL in venous blood and blood from LAA in patients with and without AF and, furthermore, compare the levels of these analytes in the two patient groups.

Methods: Samples were derived from patients with or without (controls) AF undergoing surgery for ischemic heart disease (IHD) or aortic valve disease (AVD). Until now 7 AF-patients (3 chronic and 4 paroxysmal AF) and 7 matched controls have been included. Blood was sampled from the LAA and from a central vein after thoracotomy but before systemic heparinisation, and platelet free plasma was prepared for analysis. MV were counted by nanoparticle tracking analysis (NTA). Procoagulant phospholipids were estimated by STA[®]-Procoag-PPL. TF was quantitated by an ELISA kit from Sekisui Diagnostics.

Results: MV (number and mean size), PPL and TF did not differ in blood from LAA and veins. Levels of MV and PPL in patients with

and without AF were not different, whereas TF was on average 44% higher in AF patients compared with controls in blood from both LAA and veins.

Conclusion: AF patients (with IHD or AVD) had a substantially higher level of TF in blood compared with similar controls without AF. We did not detect a difference in MV and PPL levels between the groups and found no difference between levels in LAA and veins.

Disclosure of Interest: None declared.

Non-vitamin K antagonist oral anticoagulants I

PO344-MON

Anticoagulation in patients undergoing cardioversion

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Background: Direct current cardioversion (DCCV), which is considered as the most effective treatment of atrial fibrillation, is associated with an increased risk of thromboembolism. The current recommendation is therefore to anticoagulate for 3 weeks before DCCV and 4 weeks after the procedure.

Aims: This study compares the safety & efficacy of NOACs (dabigatran) and vitamin K antagonist (warfarin) around DCCV in terms of stroke prevention, time taken to get to DCCV and the success of cardioversion in achieving sinus rhythm.

Methods: All patients referred for elective DCCV for treatment of AF in Hampshire Hospitals NHS Foundation Trust over 12 months were included in this study

Results: During a period of 12 months in 2013–14, 129 patients were referred for DCCV for the treatment of AF. DCCV was performed in 107 patients. The majority of patients (89.7%) had low CHA2DS2-VASc score between 0 and 3. Fifty four patients received dabigatran as anticoagulation for DCCV, 42 warfarin and 11 patients received other NOACs. The average time between the date of referral for DCCV and the date of DCCV for patients who were on dabigatran was 51 days whilst for warfarin this was 82 days ($P = 0.001$). The proportion of cancellation and re-scheduling to a later date was 5.5% for dabigatran but 21.4% for warfarin. The overall success of DCCV as evaluated at 6–20 weeks was 57%. The success rate for dabigatran patients was 61% whilst for warfarin patients this was 52%. The success rate for patients who received DCCV within 45 days went up to 69%. There were no reported cases of stroke and no bleeding episodes.

Conclusion: This single-centre study has shown that dabigatran is safe and well tolerated in the context of DCCV. Use of dabigatran for anticoagulation facilitated earlier DCCV than warfarin and earlier DCCV was associated with higher rates of successful restoration of sinus rhythm.

Disclosure of Interest: None declared.

PO345-MON

Evaluation of dabigatran, rivaroxaban and apixaban target-specific assays in a multicenter French study

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Background: Dabigatran etexilate, rivaroxaban and apixaban (NOACs) are widely used in prevention and treatment of thromboembolic diseases. Measurement of their concentration is desirable in certain clinical situations and target-specific assays are commercially available. However, limited information exists on the performance of these tests especially in their ability to accurately measure low and high concentrations in routine practice.

Aims: To define, in a multicenter study, the precision and accuracy of NOAC measurements in daily practice.

Methods: Fifteen blinded plasma samples (kindly provided by Hyphen Biomed) were spiked with 5 concentrations of dabigatran, rivaroxaban or apixaban (targeted 0-40-100-250-500 ng mL⁻¹ and measured by HPLC-MS/MS), and sent to 30 GEHT-laboratories. NOAC concentration, PT and aPTT were measured once in each sample using local reagents. For each sample, interlaboratory precision was determined by its coefficient of variation (CV) and accuracy by its bias. Correlations between PT or aPTT ratios and NOAC concentrations were also assessed.

Results: Four hundred and sixty four NOAC measurements were performed in the 30 laboratories using 4 reagents for dabigatran and 5 for rivaroxaban and apixaban, on 3 analysers. A modified calibration curve for low concentrations was used in 7 labs.

Interlaboratory CVs were below 18% for concentrations ≥ 100 ng mL⁻¹, and, of 29%, 25% and 16% for concentrations ~ 40 ng mL⁻¹ of dabigatran, rivaroxaban and apixaban, respectively. Biases were below 12% for all drugs and concentrations, and not lower when using a modified calibration curve. In NOAC-free samples, concentrations were all below the lower limit of quantification except for 1 dabigatran value (35 ng mL⁻¹). A strong correlation between PT, aPTT ratios and NOAC concentrations was found with the three drugs.

Conclusion: NOAC assays may allow, in daily practice, the reliable measurement of a wide range of dabigatran, rivaroxaban and apixaban concentrations with a low interlaboratory variability.

Disclosure of Interest: None declared.

PO346-MON

Validation studies on coagulation laboratory assays useful for assessing the plasma levels of Apixaban

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Background: Apixaban (A)(BMS/Pfizer), is clinically developed for DVT prophylaxis in patients undergoing hip and knee surgery, stroke prevention in patients with atrial fibrillation. It is claimed for all new oral anticoagulants that there is no need to routinely monitor A. However, there are clinical settings that would benefit from monitoring. At this time a laboratory assay for A has not yet been defined.

Aims: This collaborative study was designed to provide information on potential laboratory tests that maybe suitable for the clinical assessment of plasma levels of A.

Methods: Each day for 5 days, a standard 6 point curve in the range of 0–1.0 $\mu\text{g mL}^{-1}$ and samples A-E were run in duplicate using the Technoclone anti-Xa assay (TaXa) (Technoclone), Coamatic anti-Xa assay (Chromogenix; Instrumentation Laboratory) (CaXa) and the Prothrombin time (PT) using Innovin, Siemens).

Results: The TaXa and CaXa assays were the most accurate in determining the concentration of A in the plasma. A large standard deviation observed which was due to the variation in instrumentation between the laboratories. The PT overestimated the A concentration at ≤ 500 ng mL⁻¹ and under estimated at higher concentrations.

Conclusion: These studies suggest that the TaXa and CaXa may be used to determine the concentration of A in plasma. Each method needs to be validated on the specific coagulation instrument to be used to assess the A concentration.

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The apixaban levels as measured by various methods are shown in the following table.

Sample ng mL ⁻¹	Technochrom, ng mL ⁻¹	Coamatic, ng mL ⁻¹	Innovin PT, ng mL ⁻¹
1000	1051.50 $\pm$ 48.2	968.26 $\pm$ 38.6	362.06 $\pm$ 138.5
500	525.1 $\pm$ 32.5	540.85 $\pm$ 40.9	944.09 $\pm$ 245.4
250	239.3 $\pm$ 28.8	267.2 $\pm$ 29.7	468.5 $\pm$ 21.60
125	140.4 $\pm$ 24.6	191.16 $\pm$ 27.01	678.55 $\pm$ 147.8
30	25.5 $\pm$ 8.9	38.7 $\pm$ 14.2	155.19 $\pm$ 93.3

PO347-MON

How do real-world apixaban concentrations compare to expected concentrations?

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Background: The availability of apixaban in clinical practice, with its predictable pharmacokinetic (PK) profile brings many practical advantages to clinicians and patients alike. However the PK profile

does not negate the fact, that anticoagulant drugs are agents with a narrow therapeutic index and for some patients an element of monitoring will be required.

Aims: To describe *real-world* apixaban concentrations of patients newly commenced or switched to apixaban therapy and compare these concentrations to expected concentrations, as recommended by the British Committee for Standards in Haematology (BCSH).

Methods: Patients commenced or switched to apixaban therapy for stroke prophylaxis in the context of atrial fibrillation (Oct 2013-Oct 2014), attending the pharmacist-led anticoagulation clinic at King's College Hospital, had an apixaban concentration (variable times after last dose) drawn and determined using an anti-Xa assay, 1 month into their treatment.

Results: During the review period, 64 patients had an apixaban concentration assayed; two during the absorption phase, 27 at the peak time point, 27 during the time between the peak and trough concentration and 8 at the trough time point. The mean peak and trough results from our subjects varied considerably, compared to the targets published by BCSH: Peak_{subjects}=177 ng mL⁻¹ (SD =85) vs. Peak_{BCSH}=128 ng mL⁻¹; Trough_{subjects}=111 ng mL⁻¹ (SD =105) vs. Trough_{BCSH}=50 ng mL⁻¹. Furthermore, women prescribed the 5 mg twice a day apixaban dose tended to exhibit higher than expected apixaban concentrations.

Conclusion: Variation in apixaban concentrations is widely seen in a *real-world* population. Whether this variation has a significant effect on clinical outcomes is yet to be determined. Future work should focus on defining the thresholds for increased bleeding and thrombotic risk in the *real-world* population, as is defined for vitamin K antagonist therapy, so apixaban therapy can be optimised.

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PO348-MON

The daily practice of noacs; an observational cohort study

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Background: The use of fixed dose Non-vitamin K dependent Oral Anti Coagulants (NOACs) in daily practice may be associated with interindividual variation in activity levels. To minimize the risk of complications, laboratory tests for NOACs may be relevant.

Aims: To assess whether laboratory test for NOACs may inform dose-response relationships in daily practice.

Type NOAC	Dose	<1 month	1month	3 months	6 months	12 months
Rivaroxaban	20 mg od (n=39)	192.6 (147.8)	215.9 (134.8)	156.2 (121.6)	183.8 (130.3)	237.6 (60.8)
	15 mg od (n=6)	127.2 (148.6)	171.4 (156.1)	111.9 (168.1)		
Dabigatran	150 mg bid (n=18)	102.7 (67.3)	98.4 (78.0)	137.4 (82.8)	127.0 (81.3)	
	110 mg bid (n=8)	149.0 (109.9)	142.5 (126.1)	108.3 (45.7)		

Mean peak Levels (ng mL⁻¹) (SD) for chromogenic anti-Xa (rivaroxaban) and DTI assay (dabigatran).

Methods: An observational study including patients with non-valvular Atrial Fibrillation who start on NOACs in the Maastricht University Medical Center, Maastricht, the Netherlands. The study was ethically approved, and is ongoing. Patients are assessed at start of NOAC, 1, 3, 6 and 12 months after start, for complications of therapy (bleeding, TE) and adverse events. Kidney function is assessed and additional blood is taken for assessment of recommended screening and activity assays.

Results: So far 174 (60.1% male, 39.9% female) patients were referred. Most patients (145; 84%) had previously used VKA of which 45.7% had unstable INRs. Follow-up was agreed to by 102/174(58.6%) patients. NOACs used were: dabigatran 150 mg bid (24.9%) or 110 mg bid (14.5%), rivaroxaban 20 mg od (48%), 15 mg od (4.6%), or 10 mg od (0.6%) or apixaban 5 mg bid (0.6%). During follow-up 25 (22.4/100 patient year) total bleeding episodes were recorded, and no TE.

Conclusion: Although mean peak levels for Dabigatran as well as Rivaroxaban did not differ significantly over time, interindividual levels differed considerably. In daily practice, tailoring type and dose of NOAC based on activity levels may reduce the risk of bleeding.

Disclosure of Interest: None declared.

PO349-MON

Potent antithrombotic effect of a novel, small-molecule and direct inhibitor of factor XIA in prevention and treatment of thrombosis in rabbit model of arterial thrombosis at doses that preserve haemostasis

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Background: BMS-724296 (trans-N-((S)-1-(4-(3-Amino-1H-indazol-6-yl)-5-chloro-1H-imidazol-2-yl)-2-phenylethyl)-4-(aminomethyl)cyclohexanecarboxamide) is a potent, reversible and direct inhibitor of human and rabbit factor XIA (FXIa) with 37 °C Ki of 0.8, and 1.2 nM, respectively. It is >5000-fold selective over most of the human coagulation proteases.

Aims: This study characterized the antithrombotic and bleeding time (BT) effects of BMS-724296 in rabbits versus the direct thrombin inhibitor melagatran.

Methods: Compounds were infused IV from 30 to 60 min before artery injury or cuticle bleed until the end of the experiment in anesthetized rabbit models of electrically-induced carotid artery thrombosis and cuticle bleeding, respectively. Preservation of carotid blood flow (CBF) was used as a marker of antithrombotic effect. To evaluate whether BMS-724296 would be effective to inhibit the growth of a pre-formed thrombus, the carotid artery was injured first with electrical stimulation. Fifteen min later, the rabbits were treated with either the vehicle or inhibitor. Clotting times were measured by a commercial haemostasis analyzer.

Results: Values of antithrombotic EC₅₀ (μM) (plasma concentration that maintained CBF at 50% of control) were 0.27 for BMS-724296 and 0.15 for melagatran. Values of cuticle BT (fold-increase over con-

trol) determined at the 80% antithrombotic efficacy level were 1.26 ± 0.06 - for BMS-724296 and 6.7 ± 0.03 -fold for melagatran ($n = 6$ per group). As expected for a FXIa inhibitor, BMS-724296 increased ex vivo activated partial thromboplastin time without changing prothrombin time and thrombin time. BMS-724296, but not the vehicle, given 15 min after electrical stimulation, prevented the further reduction of CBF (an index of thrombus growth) dose-dependently, suggesting that BMS-724296 was effective in arresting the growth of preexisting arterial thrombus.

Conclusion: These results support that FXIa inhibition represents a promising antithrombotic target for the treatment of arterial thrombosis.

Disclosure of Interest: P. Wong Shareholder of: Bristol-Myers Squibb, Employee of: Bristol-Myers Squibb, M. Quan Employee of: Bristol-Myers Squibb, C. Watson Employee of: Bristol-Myers Squibb, E. Crain Employee of: Bristol-Myers Squibb, A. Rendina: None declared, J. Luetgen Employee of: Bristol-Myers Squibb, R. Wexler Employee of: Bristol-Myers Squibb, D. Seiffert: None declared.

PO350-MON

Comparing FEIBA with recombinant FVIIa for reversal of rivaroxaban with a modified anti-Xa assay in undiluted Plasma

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Background: Novel oral anticoagulants (NOAC) have better predictable pharmacokinetic property than traditional anticoagulants. Although specific antidotes for NOAC are being developed, these treatment options are not yet available for clinical use. Physicians still resort to traditional, nonspecific hemostatic agents such as Factor Eight Inhibitor Bypass Activity (FEIBA) or recombinant activated factor VII (rFVIIa) if patients on NOAC develop life-threatening bleeding. Without good quality *in vivo* trials comparing FEIBA and rFVIIa, we examined the *in vitro* reversal effects of these hemostatic agents for rivaroxaban.

Aims: With a factor (F) Xa activity assay measuring inhibitory effect of rivaroxaban, we compared FEIBA to rFVIIa in reversing rivaroxaban as determined by the normalization of FXa activity.

Methods: Normal pooled plasma was freshly defibrinated with anrod prior to use. The defibrinated plasma was incubated with 150 ng mL^{-1} rivaroxaban and 15 mM Ca^{2+} at 37°C for 15 min with or without one of the hemostatic agents being studied. The residual activity of FXa in the undiluted plasma samples was determined using chromogenic S2222 substrate at 405 nm in a spectrophotometer.

Results: In this undiluted plasma assay system, rivaroxaban inhibited FXa in a non-linear manner with the maximum effects achieved at 150 ng mL^{-1} . Rivaroxaban inhibition was reversed by FEIBA in a dose-dependent fashion. At 2 U mL^{-1} , FEIBA fully normalized FXa inhibition by rivaroxaban. Despite the ability of tissue factor (TF) to increase FXa activity, rFVIIa could not reverse the anticoagulant activity of rivaroxaban even with the addition of extrinsic purified TF.

Conclusion: In this study, FEIBA is more potent than rFVIIa in reversing the plasma FXa activity inhibited by rivaroxaban. This is consistent with the real-world experience that was published as case reports in the literature. It is also concordant on the results in our previous study using a modified Hemoclot assay for dabigatran reversal.

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PO351-MON

Discovery of ONO-7750512, an orally bioavailable small molecule factor XIA inhibitor: the pharmacokinetic and pharmacological profiles

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Background: Patients with severe factor XI (FXI) deficiency have a low risk of deep vein thrombosis. In addition, FXI antisense oligonucleotide prevented venous thrombosis in human and seems to be safe in terms of bleeding risk (N Engl J Med 2015;372:232–240). Thus, FXI is considered to be a promising drug target.

Aims: ONO-7750512 is a selective small molecule human factor XIa (FXIa) inhibitor. The objectives of this study were to elucidate *in vitro* pharmacological profile and to investigate oral bioavailability (BA) and *in vivo* antithrombotic effects of ONO-7750512 in various animal species.

Methods: To determine the oral BA, ONO-7750512 was orally or intravenously administered to rats, and serial blood sampling was performed after dosing. The plasma ONO-7750512 concentrations were measured by LC/MS/MS. The antithrombotic efficacy was assessed in a rabbit model of deep vein thrombosis induced by ferric chloride and a monkey model of arteriovenous shunt thrombosis. The hemorrhagic effect of ONO-7750512 was assessed in a rabbit model of ear bleeding.

Results: ONO-7750512 inhibited human FXIa activity with an IC_{50} value of 3.8 nmol L^{-1} . The *in vitro* plasma assessment revealed that ONO-7750512 doubled the activated partial thromboplastin time (APTT) at 1.2, 0.64, and $0.85 \text{ } \mu\text{mol L}^{-1}$ in humans, monkeys, and rabbits, however; it barely prolonged prothrombin time (PT) even at $33 \text{ } \mu\text{mol L}^{-1}$ in all the species tested. The oral BA of ONO-7750512 was 22% in rats. ONO-7750512 potentially inhibited thrombus formation at $4.0 \text{ mg kg}^{-1} \text{ h}^{-1}$ (maintenance dose) without increasing blood loss in rabbits. ONO-7750512 significantly inhibited thrombus formation in monkeys at $0.17 \text{ mg kg}^{-1} \text{ h}^{-1}$. The compound at $0.17 \text{ mg kg}^{-1} \text{ h}^{-1}$ produced a plasma concentration of 754 nmol L^{-1} and 1.9-fold APTT prolongation over control.

Conclusion: We identified ONO-7750512 as a selective human FXIa inhibitor with a fair oral BA and potent antithrombotic efficacy without increasing bleeding risk.

Disclosure of Interest: None declared.

PO352-MON

Monitoring novel anticoagulants dabigatran, rivaroxaban and apixaban using the new fully automated thrombelastography technique TEG® 6S

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Background: Novel anticoagulants NOACS dabigatran DABI rivaroxaban RIVA and apixaban APIX do not require routine monitoring.

There are situations where monitoring would be useful including bleedings, trauma, and thromboembolic events while on anticoagulation.

Aims: The purpose of this in vivo study was to assess the anticoagulant effect of NOACS with the next generation fully automated TEG[®]6S using resonance-frequency viscoelasticity measurements and disposable multi-channel microfluidic cartridge.

Methods: Nine healthy male volunteers were given single dose of oral DABI 150 mg RIVA 20 mg or APIX 10 mg. Phlebotomy performed at 0, 1 and 3 h after intake of NOAC. Reaction time R was measured using TEG[®]6S. Concentrations (conc.) of DABI, RIVA and APIX was measured using chromogenic assay Biophen[®] DTI and DiXaI. Based on available literature on pharmacokinetics of NOACS, clinical range for each agent was estimated. The TEG[®]6S R value was correlated to the drug conc. and sensitivity SN specificity SP positive and negative predictive values PPV&NPV for the set drug levels for each agent was calculated.

Results: Using the DTI channel, R was highly correlated with DABI conc. $r = 0.94$, $P < 0.0001$. R value of > 2.5 had 100% SN 90% SP, 80% PPV and 100% NPV for DABI conc. of > 50 ng mL⁻¹.

Using the AFXa channel, R was highly correlated with RIVA conc. $r = 0.93$, $P < 0.0001$. R value of > 3.4 had 100% SN, 91% SP, 94% PPV, and 100% NPV for RIVA conc. of > 100 ng mL⁻¹.

Using the AFXa channel, R was highly correlated with APIX conc. $r = 0.83$, $P < 0.0001$. R value of > 2.5 had 100% SN, 92% SP, 94% PPV and 100% NPV for APIX conc. of > 50 ng mL⁻¹.

Conclusion: We have for the first time demonstrated that TEG[®]6S has the potential of monitoring the NOACS effect on the hemostasis with highly significant correlation with the drug levels of each agent. This novel technology is easy to use on small amount of whole blood without need for specialized expertise. Larger clinical studies are warranted for correlation of TEG6S findings and clinical outcome.

Disclosure of Interest: R. Artang Speaker Bureau of: Bristol-Myer Squibb, G. Galloway: None declared, J. Amiral: None declared, J. Dalsgaard Nielsen: None declared.

PO353-MON

Enhancement of fibrinolysis by a factor XA inhibitor edoxaban and combination with an inhibitor of activated thrombin-activatable fibrinolysis inhibitor in vitro

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Background: Anticoagulants are expected to enhance fibrinolysis by inhibiting the coagulation cascade and antifibrinolytic effects of thrombin, which include thrombin-activatable fibrinolysis inhibitor (TAFI) activation.

Aims: To evaluate the effect of edoxaban, an oral direct factor Xa (FXa) inhibitor, on clot lysis using human plasma in vitro, and the combination effect of edoxaban and a TAFIa inhibitor, potato tuber carboxypeptidase inhibitor (PCI) on fibrinolysis.

Methods: Pooled human plasma containing 180 ng mL⁻¹ tissue-type plasminogen activator (t-PA) and 0.1 nM thrombomodulin were mixed with edoxaban and/or PCI. Clot formation was induced by 2.5 pM tissue factor and 4 μM phospholipids. To monitor the clot formation and lysis, the absorbance at 405 nm was measured every 30 sec. Clot lysis time was defined as the interval between the time of the midpoint of the clear to maximum turbidity transition and the midpoint of the maximum turbidity to clear transition.

Results: Edoxaban at clinically relevant concentrations of 75, 150, and 300 ng mL⁻¹ significantly shortened the clot lysis time in a concentration-dependent manner. PCI (0.3, 1, and 3 μg mL⁻¹) also accelerated the clot lysis. The combination of edoxaban and PCI significantly

reduced the clot lysis time compared with the inhibitors alone. The combination effect was additive.

Conclusion: An oral direct FXa inhibitor, edoxaban, at clinically relevant concentrations, enhanced t-PA-induced clot lysis, suggesting that this effect might contribute to the antithrombotic efficacy, especially in the treatment of venous thromboembolism. Combination of edoxaban and a TAFIa inhibitor exerted an additive effect on clot lysis, indicating that concomitant use of edoxaban and a TAFIa inhibitor might be beneficial for the treatment of thromboembolic diseases.

Disclosure of Interest: Y. Morishima Employee of: Daiichi Sankyo Co., Ltd., C. Kamisato Employee of: Daiichi Sankyo Co., Ltd., T. Furugohri Employee of: Daiichi Sankyo Co., Ltd.

PO354-MON

Novel oral anticoagulants: incidence and management of major bleeding. Retrospective review at a single center

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Background: The novel oral anticoagulants (direct inhibitors of thrombin or factor Xa) do not have yet a specific antidote to reverse its effect immediately in case of severe bleeding or emergency surgery. However its short half life suggests that only in extreme emergencies it will be necessary to reverse them. There is little evidence about the best way to manage these patients.

Aims: To describe episodes of major bleeding (ISTH bleeding scale) in patients treated with direct oral anticoagulants in our hospital between 2012 and 2014.

Methods: In our center we control about 5000 anticoagulated patients, approximately 300 patients are taking novel oral anticoagulants. A retrospective analysis of bleeding cases in these patients has been done. We have identified which patients have had major bleeding according to the ISTH scale.

Results: We included 16 patients. The average age was 75 years. 15 of them with atrial fibrillation, and 1 with recurrent deep vein thrombosis. Eleven were taking dabigatran and 5 were taking rivaroxaban. The location of the bleeding were: 9 gastrointestinal; 4 intracranial; 1 hemoperitoneum; 1 hemopericardium and 1 hematoma post arterial puncture. Eleven patients needed red blood cells transfusions and three of them got fresh frozen plasma transfusion. Two patients needed surgery. Another two patients needed invasive procedures: one arterial embolization and another one pericardiocentesis. Only in two cases specific agents were administered for the reversal of anticoagulant effects. Prothrombin complex concentrated in one patient treated with rivaroxaban, and activated prothrombin complex concentrated (Feiba) in another patient with dabigatran. All 16 episodes were favourably resolved.

Conclusion: Although nowadays we do not have specific antidotes for novel anticoagulant drugs, all cases of major bleeding that were managed in our hospital were resolved favourably with general measures and only two cases required the administration of procoagulant drugs.

Disclosure of Interest: None declared.

PO355-MON

Initiating thromboprophylaxis with rivaroxaban up to 26 h after elective knee arthroplasty does not increase the incidence of venous thromboembolism

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Background: A major complication after total knee arthroplasty (TKA) is venous thromboembolism (VTE). Various anticoagulants including rivaroxaban are used for postoperative thromboprophylaxis. Despite this modern practice, some TKA patients still develop VTE in the immediate postoperative period.

Aims: To investigate whether the time to initiate rivaroxaban prophylaxis (TTI_{RIV}) is associated with postoperative VTE in patients after TKA.

Methods: A retrospective case-control study was conducted in an academic hospital from Jan to Dec 2014. From the records of thrombosis services, we first identified patients who developed their first lifetime episode of VTE within 14 days of TKA despite rivaroxaban prophylaxis. Each index patient was matched to 6 controls with no previous VTE, who received the same post-TKA prophylaxis, and had an uneventful recovery. The duration between the end of surgery and the initiation of rivaroxaban was defined as TTI_{RIV}. Patients' demographics, medical and surgical data were also extracted for further analysis. Those receiving other thromboprophylaxis such as antiplatelet drugs, heparin, or mechanical compression within 5 days before or after surgery were excluded. The means TTI_{RAV} for both groups were compared using Student's *t*-test. A *P* value of < 0.05 was considered significant.

Results: Four VTE patients (1.7%) were identified from a total of 234 patients receiving rivaroxaban prophylaxis after TKA in this 12 month period. The mean TTI_{RIV} in the case and control groups were 27.6 ± 5.5 h and 26.4 ± 4.2 h, respectively (*P* = 0.35).

Conclusion: This study illustrates the real-world practice of rivaroxaban prophylaxis after TKA. The TTI_{RIV} in our patients were longer than the 6–8 h window period described in the RECORD trials, yet with no increase in the incidence of post-operative VTE. More, the TTI_{RIV} in our patients with VTE was similar to that in controls, suggesting patients with high bleeding risk may postpone the rivaroxaban thromboprophylaxis up to 26 h post-TKA.

Disclosure of Interest: None declared.

PO356-MON

Evaluation of the safety and tolerability of target specific oral anticoagulants at a large university setting

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Background: Multiple clinical trials have documented the safety and efficacy of target specific oral anticoagulants (TSOAC) in various patient populations.

Aims: To determine the appropriateness of prescribing patterns for the TSOACs and document the long term safety and tolerability in a real world setting.

Methods: A retrospective data analysis of our electronic health record system was conducted to obtain data for patients taking rivaroxaban (RIVA) or dabigatran (DABI) between March 2011 and July 2013. Appropriateness of prescribing was determined from prescriptions

written by providers at University of Colorado Health based on FDA approved dosing and institutional guidelines. Clinically significant hemorrhage (major bleeding or bleeding which required medication to be discontinued), major thrombosis (TIA/CVA or VTE), and other adverse drug events (ADE) were collected for all patients who had RIVA or DABI as a documented active medication during the study period and with at least 1 month of follow up. Discontinuation of medication was assessed for patient tolerability.

Results: During the study period a total 564 patients were evaluated for RIVA (*n* = 204) or DABI (*n* = 360) use for atrial fibrillation or treatment of VTE.

A total of 261 patients received new prescriptions for RIVA (*n* = 99) or DABI (*n* = 162), of which 25 (9.6%) had inappropriate dosing. Of these, 23/25 were due to under dosing of the individual agent. Clinical outcomes include:

	DABI (n=360)	RIVA (n=204)
Clinically significant hemorrhage	4.2%	6.4%
Major Thrombosis	1.4%	1.5%
Other ADE	0.5%	1.0%
Total Discontinuation Rate	29.2%	41.2%

Of patients who discontinued therapy, 38% in the DABI group and 25% in the RIVA group resumed a different oral anticoagulant.

Conclusion: Our real world analysis highlights the importance of the continual need for provider education to optimize outcomes. While the rates of ADEs, bleeding, and thrombotic events appear consistent with reported clinical trials, the rates of drug discontinuation are higher and merit further analysis.

Disclosure of Interest: None declared.

PO357-MON

Evaluation of anticoagulant therapy with dabigatran based on ecarin clotting time

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Background: Dabigatran (Dab), a direct thrombin inhibitor, has been approved for prevention of cerebral embolism in patients with non-valvular atrial fibrillation (NVAF). Coagulation monitoring is not required when Dab is administered in routine clinical practice. However, treatment for prevention of major bleeding or thrombotic events may be needed in those cases.

Aims: We examined the anticoagulant ability of Dab by measuring ecarin clotting time (ECT) and effects of anticoagulation by determining molecular markers for thrombosis.

Methods: Sodium citrate plasma samples were obtained from 452 Japanese NVAF patients receiving Dab therapy, of whom 308 received 220 mg per day (low-dose) and 144 received 300 mg per day (regular-dose). ECT was initiated by adding ecarin at a final concentration of 0.83 units mL⁻¹. To determine anticoagulation ability, thrombin inhibition ratio (TIR) was calculated using the following formula: TIR = 10 × (patient ECT – control ECT)/control ECT. Molecular markers for thrombosis were assayed using D dimer (DD) and fibrin monomer complex (FMC). Also, Dab concentration was determined with diluted thrombin time (DTI).

Results: TIR showed a greater correlation with Dab concentration determined using DTI as compared with PT and APTT. TIR values

for most of the low-dose samples were similar to those for the regular-dose samples, though low values were detected in some, suggesting insufficient anticoagulant effects. Although the frequency of high DD in the low-dose group was similar to that in the regular-dose group, the frequency of high FMC was higher in the low-dose group.

Conclusion: TIR can reflect anticoagulation ability in patients receiving Dab therapy and FMC may have a greater specificity for high coagulation than DD. Most samples from patients receiving low-dose Dab had anticoagulant effects similar to those from patients treated with regular-dose Dab, though an insufficient anticoagulant effect was detected in some low-dose samples.

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PO358-MON

Switch from vitamin k antagonists (vkas) to direct oral anticoagulants (DOACS): a practical approach

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Background: DOACs has been approved in several countries for the treatment of atrial fibrillation (AF) and venous thromboembolism (VTE) and their efficacy and safety has been shown in clinical trials. Their pharmacologic profile, which allows not routinely monitoring or dose adjustment, leading many patients to replace VKAs with DOACs. However less data are available about this switch and more studies are needed.

Aims: To evaluate the safety and efficacy of switch from VKAs to DOACs.

Methods: Consecutive patients (pts), >18 years, males and females, in treatment with VKAs for AF or VTE from Sep2013 to Dec 2014 and switched to DOACs are considered in our registry. Due to the difficulty in checking the INR immediately before starting DOACs, such as recommended, considering pharmacologic profile of VKAs and conventional bridging therapy we have established to stop warfarin 48 h before starting DOACs and acenocumarol 24 h before. Follow-up (FU) was scheduled after 3, 6 and 12 months.

Results: Three hundred and thirty one consecutive pts (54% M), mean age 68 year (range 21–92) were included in our study. One hundred and thirty nine pts (54.7% M), mean age 75 year (range 57–91) switched to DOACs were previous treated with VKAs for AF, while 192 pts (53.6% M), mean age 62 year (range 21–92) were treated for VTE. Mean time in DOACs after switch was 6.4 months (range 1–16), less for VTE pts, 5.5 months, than AF pts, 7.3 months. During first three months of FU we have reported three bleedings (2 minor and 1 clinically relevant non-major bleeding), no thromboembolic events. During following 9 months of FU no bleedings have been reported by our patients, only one myocardial infarction occurred in a patient in treatment for previous VTE and acute coronary syndrome. Two patients died, but the deaths were not related to anticoagulation treatment.

Conclusion: Usually only few pts tested the INR before to start DOACs, but if the VKAs intake is stopped 24/48 h before switch, rare adverse events occurred in the patients.

Disclosure of Interest: None declared.

PO359-MON

Warfarin and major adverse cardiovascular events in the “Real World” of patients with atrial fibrillation

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Background: Time in therapeutic range (TTR) is commonly used to assess quality of anticoagulation. TTR inversely correlates with ischemic stroke and bleeding. Few data on the relationship between TTR and myocardial infarction (MI) are available.

Aims: Our aim was to investigate the association between TTR and MI/cardiovascular death in a cohort of atrial fibrillation (AF) patients treated with vitamin K antagonists (VKAs).

Methods: We calculated TTR for 642 patients with non-valvular AF patients treated with VKAs, who were followed for a median of 28.8 months (1788.6 patients-year). The primary outcome of the study was a combined endpoint of Major Adverse Cardiovascular Events (MACE) including fatal/nonfatal MI, cardiac revascularization, and cardiovascular death. Mean age was 73.3 (±8.2) years, and 39.9% were female.

Results: During follow-up, we recorded 70 events: 18 (1.0% per year) stroke/TIA and 52 MACE: the latter included 30 MI/cardiac revascularization (1.7% per year) and 22 cardiovascular deaths (1.2%/year). The cohort was categorized according to tertiles of TTR values: TTR 17–58%, TTR 59–74%, and TTR 75–100%. There was a significant increased rate of MACE across tertiles of TTR (Log-Rank test: $P < 0.001$). On Cox proportion hazard analysis, the 2nd tertile of TTR vs. 1st ($P = 0.024$, hazard ratio [HR] 0.472, confidence interval [CI] 95% 0.246–0.906), 3rd tertile of TTR vs. 1st ($P = 0.004$, HR 0.313, CI 95% 0.143–0.689) independently predicted MACE.

Conclusion: TTR was an independent predictor of MACE in our cohort of AF patients. The incidence of MACE exceeded that of stroke, and remained elevated even in the highest tertile of TTR.

Disclosure of Interest: None declared.

PO360-MON

Global anticoagulant screening using a dilute RVV reagent

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Background: The therapeutic arsenal of anticoagulants available to clinicians has increased significantly due to the worldwide approval of direct oral anticoagulants (DOACs) like dabigatran (Pradaxa[®]), rivaroxaban (Xarelto[®]), apixaban (Eliquis[®]) and edoxaban (Savaysa[®]). Though DOACs do not require regular monitoring, their assessment in certain situations is useful.

Aims: This study is to provide a global anticoagulant screening method to assess the presence of anticoagulants without running specific anticoagulant assays.

Methods: The Global Anticoagulant Screening reagent is a lyophilized dilute RVV formulation with a high concentration of lipid. The automated Global Anticoagulant Screening assay tests anticoagulant-containing citrated plasma samples on the Instrumentation Laboratory's ACL TOP Family analyzers, quality controlled by two lyophilized plasmas (one without any anticoagulant and another with a low level of DOAC). The assay sensitivity was evaluated by testing normal pooled plasma (NPP) spiked with different levels of antithrombin-dependent anticoagulants (UFH, LMWH and Arixtra) and DOACs (dabigatran, rivaroxaban and apixaban). Furthermore, DOAC samples (50 ng/mL⁻¹, $n = 20$ for each group) were evaluated against the assay baseline established from 60 normal samples.

Results: The clotting time for anticoagulant spiked NPP increased by 173% (0.5 IU mL⁻¹ UFH), 28% (0.5 IU mL⁻¹ LMWH), 20% (0.5 µg mL⁻¹ Arixtra), 106% (50 ng mL⁻¹ dabigatran), 56% (50 ng mL⁻¹ rivaroxaban) and 21% (50 ng mL⁻¹ apixaban), respectively, versus a baseline of 45 sec for NPP. For each group of DOAC samples at 50 ng mL⁻¹, 60% (apixaban), 90% (rivaroxaban) and 100% (dabigatran) samples were above the threshold of baseline mean + 3SD. When baseline mean + 2SD is used as the cutoff, the positive percentage is increased to 75% (apixaban) and 95% (rivaroxaban).

Conclusion: The Global Anticoagulant Screening assay provides a useful research tool for the screening of anticoagulants in human citrated plasma samples.

Disclosure of Interest: None declared.

PO361-MON

A chromogenic factor XA inhibitor assay for quantifying rivaroxaban levels: optimization and validation on the ACL TOP 700

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Background: Rivaroxaban is an oral, specific, direct Factor Xa (FXa) inhibitor that inhibits free, prothrombinase-bound, and clot-associated FXa in a concentration-dependent manner, preventing thrombin generation.

Aims: To optimize and validate an automated chromogenic assay for quantifying rivaroxaban levels in human plasma.

Methods: Excess FXa is added to a rivaroxaban-containing plasma sample; residual FXa hydrolyzes a chromogenic substrate and released pNA is measured photometrically at 405 nm using the DiXaI kit (Hyphen Biomed) on the ACL TOP 700 autoanalyzer.

Results: The calibration curve consisted of 7 points using purchased rivaroxaban calibrators (Hyphen BioMed). For all runs, an $r^2 > 0.995$ was observed. To evaluate accuracy, 2 plasma samples containing rivaroxaban were compared to the value assigned by ECAT proficiency. Intra- and inter-assay precision was determined by analyzing 3 samples of various concentrations over multiple assay runs spanning multiple days. Accuracy, intra- and inter-assay precision met acceptance criteria ($\pm 15\%$ from assigned value for samples ≥ 100 ng mL⁻¹ or within 15 ng mL⁻¹ of assigned values for all other samples). Assay results were not affected by unfractionated heparin up to 1 IU mL⁻¹, but were affected by LMW heparin at 2 IU mL⁻¹. Serum and EDTA-plasma resulted in $>10\%$ difference from the citrated plasma, indicating assay interference. Analytical sensitivity and stability were established. The limit of quantitation (LoQ) was determined to be 42 ng mL⁻¹ and samples refrozen up to 2 times were acceptable. The reportable range was determined to be 42–400 ng mL⁻¹ based on the calibration curve and LoQ.

Conclusion: We have optimized and validated an accurate, precise, sensitive, and robust chromogenic assay on the ACL TOP 700 for the determination of rivaroxaban concentration in human plasma. This assay may prove useful in certain clinical circumstances (urgent surgery, severe bleeding, or thrombosis despite treatment) for the assessment of anticoagulation status.

Disclosure of Interest: None declared.

PO362-MON

Bleeding and thromboembolic outcomes in warfarin- and dabigatran-treated patients in the RE-LY trial who required an urgent surgery or procedure

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Background: There is concern about the management of anticoagulated patients with atrial fibrillation (AF) who require an urgent surgery/procedure, especially in those receiving a novel oral anticoagulant such as dabigatran etexilate (DE).

Aims: To determine rates of major bleeding (MB) and thromboembolism (TE) in patients who had an elective or urgent surgery/procedure and, separately, in patients receiving warfarin or DE, using data from RE-LY.

Methods: We assessed AF patients with a 1st treatment interruption for an urgent or elective surgery/procedure. Patients were analyzed according to type of procedure (urgent vs. elective) and by treatment (warfarin vs. DE 110 mg/150 mg). For the perioperative period (–Day 7 to Day 30, relative to surgery), rates of MB, and TE (stroke, systemic embolism, MI, vascular death, and pulmonary embolism) were determined. Multivariable logistic regression was used to assess the effect of surgery/procedure type, and the effect of treatment, on MB+TE, after adjusting for key covariates. RE-LY was approved by medical ethics committees, and written informed consent was obtained.

Results: Three hundred and fifty three and 4168 patients, respectively, had an urgent or elective surgery/procedure as defined above. Rates of MB, TE, and MB+TE were higher in patients having urgent than elective surgery/procedures, and were not significantly different in warfarin- and DE-groups. For all patients having a surgery/procedure, predictors of combined MB+TE were: urgent surgery/procedure (odds ratio [OR] = 7.49; 95% confidence interval [CI]: 5.61–10.01); heparin bridging (OR = 2.84; CI: 2.17–3.72); creatinine clearance <50 mL min⁻¹ (OR = 1.71; CI: 1.25–2.34); and age ≥ 75 years (OR = 1.35; CI: 1.02–1.78).

Conclusion: Anticoagulated patients who require an urgent surgery/procedure have a substantially higher risk for MB and/or TE than if the surgery/procedure is elective. The risk for perioperative bleeding and/or TE did not differ depending on treatment with warfarin or DE.

Disclosure of Interest: J. Douketis Consultant for: Bristol-Myers Squibb, Pfizer, Boehringer Ingelheim, AGEN Biomedical, Ortho-Janssen, Bayer, Astra Zeneca, Sanofi, J. Healey: None declared, M. Brueckmann Employee of: Boehringer-Ingelheim GmbH & Co. KG, M. Fraessdorf Employee of: Boehringer-Ingelheim GmbH & Co. KG, A. Spyropoulos Grant/Research Support from: Bayer, Astellas, Portola, Consultant for: Boehringer Ingelheim, Bayer, Johnson & Johnson, BMS, Pfizer, L. Wallentin Grant/Research Support from: Boehringer Ingelheim, Astra Zeneca, J. Oldgren Grant/Research Support from: Boehringer Ingelheim, Consultant for: Boehringer Ingelheim, Speaker Bureau of: Boehringer Ingelheim, Bayer, Merck, P. Reilly Employee of: Boehringer-Ingelheim Pharma Inc, M. Ezekowitz Consultant for: Boehringer Ingelheim, ARYx Therapeutics, Pfizer, Sanofi, Bristol Myers Squibb, Portola, Diachi Sanko, Medtronic, Merck, Johnson & Johnson, Gilead, Janssen Scientific Affairs, Astra Zeneca, Eisai, Pozen Inc, Speaker Bureau of: Boehringer Ingelheim, S. Connolly Grant/Research Support from: Boehringer Ingelheim, Sanofi-Aventis, Portola, Bristol-Myers

Squibb, Consultant for: Boehringer Ingelheim, Sanofi-Aventis, Portola, Merck, Speaker Bureau of: Boehringer Ingelheim, Sanofi-Aventis, Portola, S. Yusuf Grant/Research Support from: Boehringer Ingelheim, Consultant for: Boehringer Ingelheim, Astra Zeneca, Sanofi-Aventis, Bristol-Myers Squibb.

PO363-MON

From laboratory to clinical practice: dabigatran effects on thrombin generation and coagulation in patient samples

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Background: Dabigatran (Dabi) is not routinely monitored. However, in emergency cases quantitative assessment is required and laboratories must provide suitable tests at all hours. Little is known about Dabi effects on thrombin generation (TG).

Aims: We aimed to assess Dabi effects on TG and on a large panel of routine and specific clotting assays.

Methods: Patient samples ($n = 241$) were analyzed for functional Dabi concentrations (Dabi-TT) using a combination of the Hemoclot Thrombin Inhibitors assay (HTI[®]) and, for samples with low Dabi levels, undiluted thrombin time (TT). Results were compared with prothrombin time (PT) and activated partial thromboplastin time (APTT). In 49 samples Dabi effects were further investigated with Calibrated Automated Thrombogram (CAT[®]) for TG and with Russell's viper venom time (RVVT), prothrombinase-induced clotting time (PiCT[®]), chromogenic Anti-IIa[®] and ecarin clotting assay (ECA[®]). Fibrinogen and D-dimer were assessed to reflect the overall coagulation status of the patient. A subset of these samples ($n = 21$) were also analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results: Dabi-TT correlated with RVVT ($R^2 = 0.49$), PiCT[®] ($R^2 = 0.73$), ECA[®] ($R^2 = 0.89$), Anti-IIa[®] ($R^2 = 0.90$) and LC-MS/MS ($R^2 = 0.81$). APTT correlated curvi-linearly with Dabi-TT ($R^2 = 0.71$), but was normal in many cases (18/70) despite Dabi-TT > 40 ng/mL. There was no association between Dabi-TT and fibrinogen or D dimer levels. Increasing Dabi concentrations prolonged lag time ($R^2 = 0.54$) and, surprisingly, augmented the ETP and Peak of CAT[®] ($P < 0.001$).

Conclusion: Thrombin-specific tests measure Dabi accurately, whereas coagulation time based assays depend more on other factors. The enhanced thrombin generation in Dabi-treated patients may predict clinically relevant hypercoagulability and warrants further investigation.

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PO364-MON

The addition of idarucizumab to plasma samples containing dabigatran allows the use of routine coagulation assays for the diagnosis of hemostasis disorders

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Background: The presence of dabigatran in patients' samples impacts most coagulation assays and can therefore interfere with the confirmation of a lupus anticoagulant or with the diagnosis of hemostasis disorders developing after the initiation of the anticoagulant treatment, such as vitamin K deficiency or acquired haemophilia. Idarucizumab is a humanized antibody fragment that binds and neutralizes the anticoagulant effect of dabigatran; however it is not known if addition of idarucizumab in vitro to human plasma containing dabigatran can reverse the effects of dabigatran on these assays.

Aims: The objective of this study was to determine whether idarucizumab added in vitro to plasma samples spiked with dabigatran fully neutralises the interference of dabigatran in these diagnostic coagulation assays.

Methods: In preliminary experiments, we identified the coagulation assays sensitive to dabigatran. These assays were then carried out with normal and abnormal plasma samples spiked with 500 ng mL⁻¹ dabigatran, with or without a 3-fold molar excess of idarucizumab.

Results: The intrinsic and extrinsic factors levels were decreased by 70% and 30%, respectively, in the presence of dabigatran. These measurements were fully normalised after the addition of idarucizumab. The screen and confirm tests used for the detection of lupus anticoagulant were prolonged by dabigatran, to such an extent that the results obtained with some spiked samples falsely suggested the presence a lupus anticoagulant. Conversely, the addition of dabigatran misleadingly corrected an abnormal APC-resistance ratio. The addition of Idarucizumab also allowed the correct identification of normal and abnormal samples with both these assays.

Conclusion: The in vitro addition of idarucizumab to plasma samples containing dabigatran authorizes the use of routine coagulation assays by fully neutralizing dabigatran, thereby allowing the diagnosis of hemostasis disorders that are concurrently present in patients taking dabigatran.

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PO365-MON

Impact of dabigatran, rivaroxaban and apixaban treatment on hemostasis point-of-care testing in patients with atrial fibrillation

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Background: The rapid monitoring of new direct oral anticoagulants (DOA) -dabigatran, rivaroxaban, apixaban- might be required in specific conditions to prevent the risk of bleeding or thrombosis. As clinical application of DOAs is growing, it might be noteworthy to know their influence on the hemostasis Point-of-Care Testing (POCT).

Aims: This study was aimed for assessing DOA effect on platelet and clotting POCT parameters in atrial fibrillation patients (pts).

Methods: Citrated whole blood was taken just before (TTrough) and 3 h after the drug administration (Tmax). Blood sampling was obtained at TTrough from 110 (dabigatran), 57 (rivaroxaban) and 30 (apixaban) pts and at Tmax, from 62, 18 and 18 pts, respectively. ACT, aPTT and PT by the portable coagulometer Hemochron/Elite (ITC, USA); thromboelastometry by ROTEM system (TEM Int, Germany) and platelet CEPI and CADP Closure times (CT) by Innovance-PFA-200 system (Siemens, Germany) were performed. Dabigatran and rivaroxaban plasma levels were measured with Hemoclot Thrombin-Inhibitors and Biophen DXaI assays (Hyphen Biomed, France) on ACL TOP700 system (Instrumentation Laboratory, Italy).

Results: At TTrough, patients with normal clotting times were: ACT, 2/110 (dabigatran), 9/57 (rivaroxaban) 7/30 (apixaban); aPTT, 0/110 (dabigatran), 5/57 (rivaroxaban), 5/30 (apixaban); PT was prolonged for all. At Tmax, all tests were significantly prolonged ($P < 0.001$) except for 3/18 (ACT) and 5/18 (aPTT) patients on apixaban. Dynamic parameters of ROTEM tests (EXTEM and INTEM, ex- intrinsic pathways; ECATEM, ecarin test) were significantly prolonged ($P < 0.001$); no change was observed in CTs. Significant correlations were found between drug levels and clotting parameters (at least, $P = 0.01$).

Conclusion: DOAs exhibit distinct effects on clotting and platelet POCT. Use of POCT might provide information in emergency clinical setting to evaluate the impact of these drugs on clotting system, when rapid medical decision should be taken.

Disclosure of Interest: None declared.

PO366-MON

Anticoagulant effect of dabigatran, rivaroxaban and apixaban treatment on different laboratory hemostasis tests in patients with atrial fibrillation

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Background: The clinical use of new direct oral anticoagulants (DOA) - dabigatran, rivaroxaban and apixaban - is importantly expanding and dosing their effect may be required in specific situations.

Aims: Aim of the study was to determine the influence of DOA treatment on different hemostasis tests in patients (pts) with atrial fibrillation. Citrated blood was taken just before (TTrough) and 3 h after the drug administration a (Tmax).

Methods: Blood sampling was obtained at TTrough from 110 pts for dabigatran, 57 pts for rivaroxaban and 30 pts for apixaban; and at Tmax, from 62, 18 and 18 pts, respectively. Dabigatran and rivaroxaban plasma levels - measured with Hemoclot Thrombin-Inhibitors and Biophen DXaI assays (Hyphen Biomed, France), respectively - PT, aPTT, Protein C (PC), Protein S (PS) and aPCR, were assessed on ACL TOP700 system (Instrumentation Laboratory, Italy); whereas Diluted Thrombin Time (dTT) was evaluated on BCS platform (Siemens, USA). Light Transmission platelet Aggregometry (LTA) was performed by using ATRACT4004 aggregometer (LabiTec, Germany).

Results: At TTrough PT, and aPTT were significantly prolonged (at least, $P < 0.01$) and, similarly, at Tmax, showed a further prolongation: dabigatran, +23%, +26%, respectively [+42% for dTT]; rivaroxaban, +43% and +21% respectively; apixaban, +14% and +6% respectively; for all, $P < 0.00$). No change was observed for the other clotting tests and LTA. At Tmax, dabigatran and rivaroxaban plasma levels were significantly increased (+122% and +116%, respectively; $P < 0.000$). Significant relationships between drug plasma concentration and PT and aPTT and dTT values were observed (at least, $P = 0.01$).

Conclusion: The anticoagulant effect of dabigatran, rivaroxaban and apixaban influences only clotting tests (PT, aPTT and dTT), whereas

the inhibitory system and platelet function were not affected. The interpretation of the laboratory test might be translate into clinical decisions with efficacy and safety.

Disclosure of Interest: None declared.

PO367-MON

A novel prothrombin time (PT) method to measure all non-vitamin K dependent oral anticoagulants (NOACS)

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Background: There is a clinical need for point-of-care (POC) methods for the novel oral anticoagulants (NOACs).

Aims: For this purpose, we modified a routine POC procedure; Zafena's Simple Simon™ PT-INR, a room temperature, wet-chemistry PT of the Owren-type (i.e. a combined thromboplastin which allows extensive dilution of sample).

Methods: To either increase or decrease NOAC-interference, two assay-variants were devised by replacing the standard 10 µL end-to-end capillary used to add sample to 200 µL of PT-reagent by either a 20 µL or a 5 µL such capillary. Both assay-variants were calibrated to show correct INR-results in plasmas devoid of NOACs from healthy and warfarin treated persons.

Results: For plasmas spiked with NOACs, the 20 µL-variant showed markedly higher INR-results than the 5 µL, the effects were more pronounced at room temperature than at +37 °C. Additional experiments were with plasmas from patients treated with dabigatran, apixaban or rivaroxaban ($n = 30$ for each). INR-values of these plasmas were compared to NOAC-concentrations determined by established methods. For all NOACs, a strong correlation was found between NOAC-levels and the INR-response of the 20 µL-variant, linear correlation coefficient (r) averaged 0.90, the INR-range was 1.1 to 2.1 for dabigatran and apixaban, and 1.1 to 5.0 for rivaroxaban. Correlations were weak and ranges narrow for the 5 µL-variant. In general, the INR-ratio between the 20 and 5 µL variants made the NOAC-assay more robust, eliminating confounding warfarin-effects and improving correlations to established NOAC-methods, all without sensitivity loss. Detection limits remained at 80 µg L⁻¹ for apixaban and dabigatran, and at 40 µg L⁻¹ for rivaroxaban. The imprecision of the method was clinically acceptable for all NOACs at therapeutic levels. For rivaroxaban the CV was 5.6% at 240 µg L⁻¹.

Conclusion: In conclusion an established wet-chemistry POC PT-INR procedure was modified to measure the concentrations of all three NOACs currently in clinical use.

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PO368-MON

Effects of new oral anticoagulants on fibrin-network permeability and thrombin generation

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Background: New oral anticoagulants (NOACs) effectively reduce the risk of ischemic stroke and systemic embolism in atrial fibrillation (AF). There are important pharmacological differences between each NOAC, and a deeper knowledge of how these different drugs influence hemostasis is desirable.

Aims: The aim of this study was to further explore the effects of the direct thrombin inhibitor dabigatran and the two Xa-inhibitors apixaban and rivaroxaban on fibrin network permeability and thrombin generation in "real-life" AF-patients.

Methods: Patients were treated with apixaban ($n = 20$), rivaroxaban ($n = 26$), or dabigatran ($n = 23$) according to clinical guidelines. For comparison we also investigated a group of patients on PT-INR adjusted warfarin treatment ($n = 27$). Fibrin network permeability (Ks) and thrombin generation were measured in samples with known plasma concentration (by liquid chromatography-tandem mass-spectrometry, LC-MS/MS) of each NOAC.

Results: Dabigatran had the strongest effect on Ks with highly significant increments compared to both warfarin and the Xa-inhibitors ($P < 0.001$). Warfarin caused higher Ks than both rivaroxaban ($P < 0.05$) and apixaban ($P < 0.001$). All drugs increased lag-time; the most pronounced effect was caused by dabigatran ($P < 0.001$ compared to both Xa-inhibitors). There was a significant correlation between lag-time and LC-MS/MS for both dabigatran ($P < 0.01$) and apixaban ($P < 0.01$) treated patients. A significant positive correlation was also seen between Ks and lag-time in dabigatran ($P < 0.01$) and warfarin ($P < 0.05$) treated patients, but not in patients on Xa-inhibitors.

Conclusion: In "real life" AF patients dabigatran treatment has stronger effects on fibrin permeability and thrombin lag-time than treatment with rivaroxaban, apixaban or warfarin. Plasma drug concentrations of NOACs show clear-cut relationships to lag-time but are not correlated to fibrin permeability. Further studies are needed to understand differential effects of NOACs on different parts of the hemostatic system.

Disclosure of Interest: None declared.

PO369-MON

Knowledge of the new oral anticoagulants (NOACs) amongst University teaching hospital doctors

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Background: In 2012/13 NICE published recommendations for the management of atrial fibrillation and venous thromboembolism with the NOACs. NICE emphasise that clinicians have a duty to help patients make decisions about their treatment based on an understanding of the likely benefits and risks of therapy.

Aims: To assess the knowledge of doctors around 3 NOACs; Dabigatran, Rivaroxaban and Apixaban.

Methods: 'On the spot' questionnaires were randomly distributed to doctors of varying grades and specialities. Data collection was discontinued on completion of 100 questionnaires. Questions included mode of action, indications, adverse effects, contraindications, monitoring, reversal and peri-operative management.

Results: Totally 35% of questionnaires were answered by senior grades;Registrar/Consultant, and 65% by juniors across Medical/Surgical specialities. All were aware of the 3 NOACs, but less than half could state individual mode of actions (39%). 46% could state NICE approved indications and 48% recognised the need for variant dosing. 47% stated prescribing contraindications;bleeding (20/47) most common followed by metallic heart valves (10/47), renal failure (9/47). 55% were aware of reasons including patient choice and a labile INR for starting a NOAC over warfarin. The majority could not state any possible adverse effects other than bleeding (66/70). 83% answered NOACs do not need monitoring,74% identified the need for interruption pre-invasive procedures/surgery. 81% reported lack of a reversal agent. Only 36% had prescribed a NOAC with Rivaroxaban (25/36) the most popular, but only 28% felt confident to initiate a NOAC. No noticeable differences were found comparing junior vs. senior doctors ($P = 0.05$).

Conclusion: A significant proportion of clinicians, including senior doctors, are unaware of vital NOAC prescribing information essential to ensure patient safety. This study highlights the need for wider education and trust policies to allow appropriate and safe prescribing.

Disclosure of Interest: None declared.

PO370-MON

Real-life use of non-vitamin k antagonist oral anticoagulants in comparison with vitamin k antagonists for non-valvular atrial fibrillation: data from a prospective cohort

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Background: Current guidelines recommend non-vitamin K antagonist oral anticoagulants (NOACs) or vitamin K antagonists (VKAs) for patients with non-valvular atrial fibrillation (NVAF). Clinical features of patients prescribed with NOACs or VKAs in real-life remain undefined.

Aims: To evaluate differences between NVAF patients prescribed with NOACs or VKAs in real-life.

Methods: Consecutive patients with NVAF prescribed with NOACs from August 2013 to January 2015 were included in a multicenter prospective cohort and compared with a random sample of patients with NVAF receiving VKAs. Chi-squared test and Student' t-test were used.

Results: Overall, 1494 patients with NVAF (5.4% new detected, 17.3% paroxysmal, 8.8% persistent, 68.5% permanent) were included: 1021 receiving NOACs (reduced doses in 483 patients) and 473 VKAs. The mean CHA₂DS₂VASC score was higher in patients treated with rivaroxaban (4.6 ± 1.5) or apixaban (4.5 ± 1.5) compared with patients treated with dabigatran (4.0 ± 1.5 ; both $P < 0.001$) or VKAs (4.1 ± 1.4 ; both $P < 0.001$). Similarly, the mean HASBLED score was higher in patients treated with rivaroxaban (3.1 ± 1.1) or apixaban (3.1 ± 1.0) as compared with patients treated with dabigatran (2.5 ± 1.2 ; both $P < 0.001$) or VKAs (2.4 ± 1.0 ; both $P < 0.001$). These differences were mostly accounted for by a lower mean age in dabigatran patients (77 ± 8 years) compared to rivaroxaban, apixaban or VKAs patients (79 ± 9 , 79 ± 8 and 79 ± 8 years, respec-

tively) and by a higher prevalence of previous stroke, previous major bleeding and labile INR in patients prescribed with NOACs compared with VKAs. Patients prescribed with reduced doses of NOACs have increased CHA₂DS₂VASC or HASBLED compared to patients treated with VKAs or standard doses of NOACs.

Conclusion: Patients prescribed with rivaroxaban or apixaban in real-life have a higher thrombotic and haemorrhagic risk in comparison to patients receiving dabigatran or VKAs. A considerable proportion of patients received reduced doses of NOACs in real-life.

Disclosure of Interest: None declared.

PO371-MON

The modulation of prothrombinase lifetime by rivaroxaban

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Background: Rivaroxaban is a direct factor Xa (FXa) inhibitor that reversibly targets both free FXa and FXa complexed with its cofactor, Factor Va, in the prothrombinase complex (PTase). Cessation of rivaroxaban treatment without administration of another anticoagulant may lead to an increased risk of stroke and other thrombotic complications.

Aims: To assess the impact rivaroxaban occupancy of PTase under flow has on the ability of antithrombin (AT) to inhibit PTase.

Methods: Prothrombinase was assembled within phospholipid-coated glass capillaries and the following sequence of reaction mixtures flowed (100 s⁻¹) through each capillary: 1) prothrombin (PT), 2) an anticoagulant solution or PT alone, and 3) PT. The first and third phases (PT alone) were used to indicate the amount of functional prothrombinase present. The anticoagulant solutions contained either AT, rivaroxaban (2.5, 10, 25 nM), or AT plus rivaroxaban (2.5, 10, 25 nM); each anticoagulant solution was also tested in the presence of PT. Immobilized PTase was exposed to the anticoagulant solutions for ~9 min. The selected rivaroxaban concentrations reflect the free C_{max}, C_{mid}, and C_{min} plasma concentrations (20 mg/od).

Results: Under flow, when the anticoagulant solutions contained AT or AT + PT, residual PTase levels averaged 50%. In the absence or presence of PT, rivaroxaban protected PTase from AT inhibition.

Conclusion: Pharmacologically relevant concentrations of rivaroxaban extend the lifetime of the prothrombinase complex in the presence of a stoichiometric inhibitor of factor Xa, supporting the potential for thrombus-associated prothrombinase to be unmasked upon cessation of rivaroxaban therapy.

Disclosure of Interest: M. C. Bravo: None declared, T. Orfeo: None declared, M. Gissel: None declared, E. Lavoie: None declared, L. Haynes: None declared, K. Mann Shareholder of: Haematologic Technologies, Consultant for: Baxter, Diagnostica Stago, Bayer, CSL Behring, Alnylam.

PO372-MON

Pharmacokinetics and -dynamics of dabigatran etexilate and rivaroxaban in patients: requiring parenteral nutrition for short bowel syndrome (the PDER PAN study)

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Background: Patients receiving parenteral nutrition (PN) for short bowel syndrome (SBS) often receive anticoagulation for prevention or

treatment of thrombosis. Limited and variable enteral reabsorption of vitamin K antagonists often precludes their use. The route of administration of heparins is burdensome and may lead to poor compliance. The rationale for dabigatran etexilate and rivaroxaban administration in patients with SBS is based on the ease of oral administration and a significant absorption in stomach and proximal bowel, which is usually maintained in patients with SBS.

Aims: The aim of this phase I study is exploring pharmacokinetics and pharmacodynamics (PK/PD) parameters of rivaroxaban and dabigatran etexilate in adult patients with SBS on home long-term PN with an investigator-initiated, single center, randomized, open-label, cross-over, phase I study.

Methods: Inclusion criteria were: clinically stable patients (18–75 years, 50–110 kg), remaining bowel length <160 cm after Treitz ligamentum, estimated glomerular filtration rate >50 mL min⁻¹, Child-Pugh score A, no recent major bleeding or thrombotic event, no co-medications interfering with study drugs, no prior gastrectomy. On Day 0 patients were randomized to receive either once-daily rivaroxaban 20 mg or twice-daily dabigatran 150 mg from Day 0 to Day 4. Relevant drug-specific PK/PD parameters were measured at different timepoints on Day 0 (single-dose curves) and on Day 4–5 (steady-state curves). After a wash-out period, patients were switched to the other anticoagulant drug and the procedure was repeated. Values were compared to available data from the literature. Patients' informed consent was obtained; the study was approved by hospital ethical committee (www.trialregister.nl; identifier, NTR4192).

Results: Four patients completed the study.

Conclusion: Final results will be presented at the congress.

Disclosure of Interest: S. Barco: None declared, Y. Cheung: None declared, M. Coppens Grant/Research Support from: Boehringer Ingelheim, Sanquin Blood Supply, Consultant for: Boehringer Ingelheim, Daiichi Sankyo, the alliance of Bristol-Myers Squibb and Pfizer, Sanquin Blood Supply, Speaker Bureau of: Boehringer Ingelheim, Daiichi Sankyo, the alliance of Bristol-Myers Squibb and Pfizer, Sanquin Blood Supply, M. Serlie Grant/Research Support from: TEFA Mediq, Consultant for: Fresenius, R. Mathot: None declared, S. Middeldorp Grant/Research Support from: Glaxo SmithKline, Aspen, Bristol-Meyers Squibb/Pfizer and Sanquin Blood Supply, Consultant for: from Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Pfizer, Daiichi-Sankyo.

PO373-MON

Is it possible to assess rivaroxaban activity without specific calibrators?

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Background: Rivaroxaban, a direct and specific factor Xa inhibitor has been approved for management of thromboembolic disorders without routine monitoring, but there are several clinical settings in which measurement of the anticoagulant activity can be helpful. Chromogenic anti-factor Xa activity (aXa) using Rivaroxaban calibrators is the preferred clinical assay, however commercial calibrators are not always available. Low molecular weight heparin (LMWH) calibrators could be used for this purpose.

Aims: To determine the correlation between aXa, expressed in ng mL⁻¹ (aXa-R), and aXa expressed in IU mL⁻¹ (aXa-IU), using Rivaroxaban and LMWH calibrators respectively, in patients receiving Rivaroxaban and secondly to correlate PT and APTT with aXa.

Methods: Ten patients taking 15 or 20 mg of Rivaroxaban once daily had two blood samples taken, at peak and at trough. Plasma

was employed to assay PT, APTT and aXa using Rivaroxaban and LMWH calibrators. Statistical analysis was performed with ANOVA test, correlation and linear regression with Barlett sphericity.

Results: We found a statistically significant correlation between aXa-R compared to aXa-IU ($r = 0.76$), this correlation was stronger at trough ($r = 0.96$) than at peak ($r = 0.69$). The correlation between PT (sec) and aXa-R was 0.88 and 0.77 at peak and at trough, respectively but 0.42 and 0.88 when compared with aXa-IU. By contrast, a weaker correlation was observed with APTT.

aXa-R, aXa-IU and PT results were statistically different between peak and trough ($P = 0.002$, 0.0005 and 0.008 respectively). Two patients at peak had PT values close the normal range.

Conclusion: Rivaroxaban anticoagulant activity with aXa using LMWH calibrators, available for routine use in clinical laboratories, provides a reliable assessment of Rivaroxaban, better at trough than at peak, allowing the use of aXa in most clinical laboratories. While PT correlates with aXa-R, it is not sensitive enough to determine drug exposition, some degree of anticoagulant cannot be excluded by a normal PT.

Disclosure of Interest: None declared.

PO374-MON

A new assay for the measurement of rivaroxaban concentrations in human plasma

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Background: Rivaroxaban (RIV) a.k.a. *Xarelto* is an oral direct FXa inhibitor. Although fixed dose without routine monitoring is indicated, in a number of situations determination of RIV levels is desirable for clinical decision.

Aims: This study was designed to develop a liquid reagent-based RIV assay with specific RIV Calibrators and Controls for rapid and accurate determination of RIV levels in citrated human plasma on ACL analyzers.

Methods: The RIV assay is built on the principle of Xa-dependent chromogenic detection with the HemosIL Liquid Anti-Xa Kit reagents. The automated 5-point calibration curves was produced using lyophilized RIV Calibrators 1 and 2 (0 and 500 ng mL⁻¹ respectively) and validated using the bi-level RIV Controls (80 and 300 ng mL⁻¹). Linearity, LoD, precision/accuracy, interference, and method comparison studies were performed according to the CLSI guidelines. On-board and reconstituted stabilities (2–8 °C, -20 °C) were measured for the plasma components.

Results: The precision of the calibrators and controls were $\leq 5\%$ CV and RIV recoveries within $\pm 10\%$ of target values. The LoD was 6 ng mL⁻¹, the test was linear between 15–550 ng mL⁻¹ and up to 1000 ng mL⁻¹ with reflex testing. No interference was observed for Hemoglobin (550 mg dL⁻¹); bilirubin (24 mg dL⁻¹); triglycerides (1100 mg dL⁻¹). The RIV Calibrators and Controls were stable for 8 h on-board, 7 days at 2–8 °C, and for 2 months at -20 °C with a single freeze/thaw. The outcome of Method Comparison was the following: a) Clinical trial: IL vs. Hyphen DiXaI: $R = 0.97$ and slope = 0.99; IL vs. HPLC-MS/MS: $R = 1.0$ and slope=1.02; b) POP studies: IL vs. Hyphen DiXaI $R = 0.99$ and slope = 0.93.

Conclusion: The IL HemosIL RIV Assay on ACL TOP is reliable and accurate for measuring RIV in citrated plasma. It has good sensitivity in the low range to verify clearance of RIV prior to surgery, as well as sensitivity in the clinical and high range. The test has satisfactory component stability, good sample precision, and comparison to a predicate device.

Disclosure of Interest: None declared.

PO375-MON

Monitoring vitamin k antagonist treatment: interests of good therapeutic education

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Background: The Vitamin K antagonists (VKA) are anti thrombotic used in the prevention and treatment of thromboembolic events in patients with heart disease. Because of its potential iatrogenic, it was decided to create an educational foundation with a study in the cardiology department of the Nafissa Hamoud Hospital.

Aims: The program is developed by searching the motivation of patients about their treatment VKA during 2 months. The patients are followed by analysing the stability of the International Normalized Ratio (INR)

Methods: Overall 289 patients were concerned, 176 females and 113 males, with a 0,3 sex ratio. We have to assess patients'knowledge about their VKA treatment through a binary language questionnaire, to obtain easier analysis leading to a yes-no answer or a true-false answer. And then, we evaluate the INR test.

Results: It has been shown that some points in the domain of knowledge to deal with situations of overdose, forgetfulness...remain unknown. In view of these results, a targeted education session was offered to patients using methods appropriate to the achievement of our goals (pictorial support, new boxes of medicines...). In the end, an evaluation of the educational process in medical term (the INR stability is the end point in our study).The result was very encouraging, indeed, 2 months later, 62% of the INR is balanced after the education session against 42% before the session.

Conclusion: An improvement of INR stability was observed 2 months after patients' education, this study suggests the importance of integrating the therapeutic educational sessions on drug with high iatrogenic risk treatments.

Disclosure of Interest: None declared.

Nursing and Allied Health I

PO376-MON

How participating in a clinical trial has had a far reaching effect – a case study

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Background: Clinical trials are an integral part of haemophilia management and with such a small global population it is imperative that Haemophilia Treatment Centres (HTC) and patients understand how participating in clinical trials can have far-reaching effects.

Aims: This case study focuses on a 19 year old male with severe haemophilia B who participated in a recombinant factor IX extended half life phase 3 clinical trial and how this participation affected him, the HTC and government jurisdictions.

Methods: A review of patient interactions/case notes and correspondence with HTC staff provided the data for this case study.

Results: Clinical Patient Outcomes: Pre clinical trial dose rFIX 5000 IU $\times$ 3/week (241 IU kg⁻¹ per week). Study commencement dose 3500 IU (50 IU kg⁻¹ per week). Dose reduced to 2000 IU (30 IU kg⁻¹ per week) and then further reduced to 1500 IU (20 IU kg⁻¹ per week) (1500 IU). Now into his 4th year of being on the clinical trial he remains on 1500 IU weekly and has reported only one bleeding event.

Psychosocial Patient Outcomes: The frequency of contact with the HTC during the clinical trial period improved the relationship and trust between HTC staff and the patient. The patient also gained a greater understanding of his bleeding disorder which gave him a

greater sense of trust, ownership and control over managing his bleeding disorder.

HTC Outcomes: The HTC has gained a greater understanding of the product being trialled and how best the product could be used within their patient population.

Government Jurisdiction Outcomes: Whilst patients are participating in clinical trials the financial impact of funding the clotting factor concentrate lies with the sponsor, which can significantly reduce the financial burden of funding clotting factor concentrate.

Conclusion: Not only did this patient gain early access to new innovations in haemophilia treatment and an enhanced quality of life, but the HTC and government jurisdictions also benefited from patient participation.

Disclosure of Interest: None declared.

PO377-MON

Barriers to inform hemophilic carriers of that possibility in Japan

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Background: Prophylactic replacement therapy in patients with hemophilia has enabled to improve their QOL. However, insecurity of hemophilic carriers at the time of marriage or pregnancy has not been resolved yet because their genetic anomaly may be inheritable. Furthermore, hemophilic carriers have not been recognized as the persons who receive health care service in Japan.

Aims: The purpose of this study is to clarify barriers to inform hemophilic carriers of that possibility in the family of possible carriers.

Methods: The subjects were mothers who have hemophilic boys and daughters who were possible carriers. We performed an investigation using the semi-structured interview to the subjects and confirmed respective hereditary of hemophilia by family trees. Study Period is from October to November in 2014.

Results: Four mothers became the subjects and their ages ranged from 30's to 50's. Two were persons who had known the possibility of bearing hemophilic boys before their marriage. And then, both of them had brothers with hemophilia. As a common opinion about notification to carriers, a category that I would like to inform them before marriage or delivery properly was extracted. It included as a subcategory that I could not inform them though notification was necessary (subcategory A) and I planned the opportunity of the notification. Subcategory A was consisted of "obscurity of appropriate notification time" "vague anxiety" "anxiety by the lack of the parents' knowledge about heredity". As promising support from specialists, they hoped that "medical care givers who specialize in hemophilia and heredity" "hospitals which can be examined anytime" "support both delivery and treatment of the patients" "stories of the carriers' experiences about diagnosis".

Conclusion: We considered that total and wide-ranged support was necessary. However, in the present state, total support of carriers may be difficult in Japan. Therefore, it is an ideal that persons who need support can choose and utilize the necessary support.

Disclosure of Interest: None declared.

PO378-MON

Mechanical thromboprophylaxis – solving dilemmas

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Background: Anti-embolism stockings (AES) and intermittent pneumatic compression devices (IPC) are commonly used methods of mechanical thromboprophylaxis (MT) for prevention of Venous Thromboembolism (VTE). Despite the frequency of their use much uncertainty exists around the practical aspects of their application. The ambiguity arises from the need to identify the risk and benefit ratio for each patient to ensure risk of harm is minimised and the potential for benefit is maximised.

Aims: We aimed to provide evidence-based solutions to the most commonly reported dilemmas by examining the available literature. In particular, our goals were to discover the optimum length of time AES should be worn, if AES cause skin damage and in which instances thigh length IPC would be chosen over knee length.

Methods: A comprehensive literature search was conducted and the results reviewed.

Results: The literature was often sparse and on some occasions conflicting. When used in conjunction with national guidance however, we found that the literature helped to construct answers to the most common dilemmas; although these answers were mostly based on current best practice rather than clinical evidence.

Conclusion: Although individual clinical judgement must be applied when making decisions about the use of AES and IPC, current literature offers some guidance to help clinicians make the right choice. This information should be shared with clinicians to ensure disparities in practice are reduced, and to improve confidence in clinical decision-making.

Disclosure of Interest: None declared.

PO379-MON

The nurses role in idiopathic thrombocytopenia

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Background: Patients with Immune Thrombocytopenia (ITP) were previously monitored by the medical doctors in the clinic. Presently, I run an independent Nurse-led clinic on ITP in my Trust. I have a very important role in the monitoring, dosing of medications and follow-up of these patients medications. The clinic is run once per week and a maximum of ten patients are seen in the Nurse Specialist clinic.

Aims: To establish continuity of care for this patient population. These patients' would have a Nurse Specialist and a specific point of contact for queries or any other issues relating to their condition.

Methods: Patients are referred to the Nurse Specialist by the Haematology Consultant or the Haematology Registrar once the patient is suspected of having ITP or a low platelet count. Patients are then booked into the Nurse Specialist clinic and followed up on a regular basis.

Results: My clinic consist of both male and female. The age range from 23 years to 82 years. The treatment types varies to steroids, azathioprine, mycophenolate, romiplostin or monoclonal antibody or immunoglobulins. From observation patients appreciate having a Nurse Specialist as it provides them with continuity of care and someone who knows their illness well and a specific point of contact. Patients also develop confidence and trust in the Nurse Specialist due to seeing the same health care professional most of the time.

Conclusion: Patients with ITP are closely monitored and followed up in the Nurse Specialist ITP clinic. The patients are long term and maybe on different types of treatment. As the Nurse Specialist, it is my responsibility to ensure patients are followed up and monitored in a

safe and timely manner. A relationship of trust and confidence is fostered between both patient and Nurse Specialist.

Disclosure of Interest: None declared.

PO380-MON

The impact of footwear on ankle arthropathy in patients with hemophilia

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Background: Despite improvements in hemophilia treatment and the use of prophylaxis, people with hemophilia (PWH) continue to experience ankle hemarthrosis, arthropathy, pain and disability. While the use of orthotics has been seen to improve ankle arthropathy for many, orthotics are not always practical or available for every patient. Questions remain about the impact of typical daily footwear for the prevention or improvement of arthropathy.

Aims: To perform a literature review investigating the impact of footwear on ankle arthropathy in PWH.

Methods: The National Guideline Clearinghouse, NICE Clinical Guidelines, Canadian Hemophilia Society, National Hemophilia Federation, World Federation of Hemophilia, and Centers for Disease Control were searched for practice guidelines and the Cochrane Library was searched for systematic reviews. Electronic databases PubMed, Ovid, and CINAHL (1947–2014) were searched. Search terms were “hemophilia,” “haemophilia,” “footwear,” “shoes” using search limits of “English language” and “human.”

Results: Review of the literature located only one study by McLaughlin et al. investigating footwear in PWH. Using gait analysis McLaughlin et al. demonstrated increased ankle joint force in both new neutral-soled running shoes and worn hard-soled shoes as compared to the same analysis in healthy controls. Also, in PWH there was a significant increase in ankle joint force at midstance when wearing the running shoe as opposed to the hard-soled shoe and hypothesized that the running shoe allows for increased muscle activity and therefore increased joint stability and less reported pain. However objective data demonstrating enhanced stability or any impact on risk of injury or pain was not provided.

Conclusion: Despite ongoing improvements in medical care and physiotherapy for patients with hemophilia, there is a noteworthy lack of available information regarding footwear. To decrease ankle arthropathy and improve outcomes, more research is needed on the impact of typical daily footwear for PWH.

Disclosure of Interest: None declared.

PO381-MON

Development of inpatient pediatric anticoagulation management service: the advanced practice nurse practitioner service model

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Background: Pediatric anticoagulation management has become increasingly complex especially in large centers. As a result, development of dedicated pediatric anticoagulation services has been advocated. Various service models have been utilized [hematologist, pharmacist, or advanced practice nurse practitioner (APNP)-directed

anticoagulation models]. Inpatient care provided by APNP has been demonstrated to be high-quality and cost-effective.

Aims: Report our center's experience with APNP-directed anticoagulation service.

Methods: In 2013, a dedicated pediatric anticoagulation service was created to provide standardized and effective management at the Children's National Health System in Washington, DC. The service is managed by inpatient APNP in consultation with 3 pediatric hematologists and is supported by a full-time nurse coordinator. To evaluate the performance of the service, we queried the children's national administrative database using The International Classification of Diseases, Ninth Revision (ICD-9) codes for thromboembolism over a 2-year period between December 2012 and December 2014. We then identified all cases managed by either the general hematology service (December 2012-December 2013) or the APNP-directed anticoagulation service (December 2013-December 2014).

Results: In the earlier year (December 2012-December 2013), 58 inpatients were managed by the general hematology service. In comparison, 96 inpatients were managed by the APNP-directed anticoagulation service in the subsequent year (December 2013-December 2014). Moreover, the total number of consults and total medical billing for inpatients increased over the same time periods (275 consults and \$37 552 compared to 763 consults and \$104 699, respectively).

Conclusion: The establishment of a dedicated anticoagulation management service directed by APNP resulted in significant growth in the number of followed patients, consultations and medical billing.

Disclosure of Interest: None declared.

PO382-MON

First experience with the haemoassist, an electronic substitution diary that works on the basis of a personal digital assistant (PDA)

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Background: Home treatment in haemophilia allows patients and caregivers to be less dependant on hospital care or haemophilia centres. It can increase independence and an earlier treatment of bleeds. Patients which are eligible for home treatment may have a more normal life. In accordance with §14 of the German Transfusion act, patients are obligated to document the factor substitution. Usually this is done by a paper based substitution calendar. The Haemoassist™, an electronic substitution diary that works on the basis of a Personal Digital Assistant, offers another option for the documentation of factor consumption for haemophilia patients in Germany since 2007.

Aims: NA.

Methods: The Haemoassist™ is an electronic patient diary to record details of factor VIII infusions for prophylaxis or on demand treatment. FVIII administration with total dose, time of infusion, brand name, lot number, reason for infusion bleeding with bleeding location has to be documented by the patient or the caregivers. The treatment centre has access to the patients data and the internet based documentation. The system sends an alarm message in predefined critical situations like three haemorrhages within 1 week or more than 6000 IU of clotting factor administered per haemorrhage.

Results: Within the last 2 years 33 patients switched to the Haemoassist™. Only 8 returned to their paper based diary because of personal discomfort or changes in their electronic equipment. Unusual substitution can be recognized fast and early information of recurrent bleedings helps to prevent further joint damage and to optimize the therapy. The compliance in using the Haemoassist™ is similar to the paper based documentation.

Conclusion: The physicians receive informations about suspicious bleeding quickly and they can control the therapy compliance easily. For our patients the Haemoassist™ is easy to use. But elderly patients seem to be not qualified for using it as this generation is mostly not so experienced in the use of modern electronic devices.

Disclosure of Interest: None declared.

PO383-MON

An evaluation of a myeloproliferative neoplasm specialist nurse led clinic

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Background: Patients with myeloproliferative neoplasms were historically seen by the Haematologists. Nonetheless, recent developments have seen this patient population being monitored by the Clinical Nurse Specialist in a myeloproliferative neoplasms clinic. I, independently consult in a myeloproliferative neoplasm Specialist Nurse -Led clinic in my National Health Service Trust. The clinic was started approximately 7 years ago in the Trust. The clinic is conducted twice per week. To establish the effectiveness of this Nurse -Led clinic, I carried out a patient satisfaction survey with a questionnaire developed for this purpose.

Aims: The objectives of this evaluation were to establish patients' perspectives about being seen in a Specialist Nurse -led clinic. In addition, this was to determine whether the patient considers a Specialist Nurse adequately skilled and knowledgeable, and does not cause feelings of anxiety.

Methods: A quantitative study design was used in the form of a questionnaire and random selection. This questionnaire comprised of twelve questions. The questionnaire was given out to patients on the day of their clinic appointment and they were asked to complete the questionnaire. Both male and female participants were included and the responses were anonymous. A total of 80 questionnaires were given out and 75 were returned.

Results: Overall 97.3% felt comfortable being seen by the Specialist Nurse. 88% were happy for the Clinical Nurse Specialist to prescribe their medications. 96% felt that the Clinical Nurse Specialist has a good understanding of their condition. 90% felt they were comfortable to ask questions.

Conclusion: Overall, patients were very satisfied at being seen by a Specialist Nurse in an independent clinic setting. The clinic has expanded over the 5-year period and a second clinic was started at another Hospital in my Trust. Since then, I have started another Nurse -led clinic in the Trust. I am in the process of setting up a telephone clinic in my hospital.

Disclosure of Interest: None declared.

PO384-MON

Pharmacist supported anticoagulation management clinic - improving patient care in an emerging country

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Background: Joint Commission International Accreditation (JCIA) outlined the National Patient Safety Goal related to anticoagulation therapy (03.05.01). We observed 10% of drug overdosage was due to warfarin toxicity.

Aims: The objective of this study is to observe improvement in patient care during anticoagulation therapy.

Methods: We studied our patients enrolled in Anticoagulation Clinic from March 2013 to December 2014. Quality indicators were monitored as bleeding and thrombotic events. Food consultation and patient education was done to 100% patients. 15 patients migrating to other cities were communicated through e-mails and telephonic messages for *INR monitoring* and *dose adjustments*.

Results: A total of 105 patients (49 males/ 56 females) were registered in Anticoagulation Clinic. Diagnosis included deep venous thrombosis ($n = 23$), pulmonary embolism ($n = 18$), portal vein thrombosis ($n = 7$), superior mesenteric vein thrombosis ($n = 5$), cerebral venous thrombosis ($n = 5$), others ($n = 47$). During the study of 105 patients 2 events were reported. These events included incidents of minor ($n = 1$) and major bleeding ($n = 0$) and thrombotic event ($n = 1$) despite anticoagulation.

Conclusion: The results showed improved patient care for bleeding and thrombotic events.

Disclosure of Interest: None declared.

PO385-MON

Nursing management of venous thromboembolism (VTE) treatment with rivaroxaban at the university hospital of Padua: a 6-month experience

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Background: Rivaroxaban is a novel oral direct factor Xa inhibitor approved in EU and USA for VTE treatment and for VTE recurrence secondary prevention. The role of physicians in managing rivaroxaban is well established. Data about the nursing management of VTE treatment with rivaroxaban are lacking.

Aims: Report the nursing experience at the Unit of Angiology of the University Hospital of Padua with the use of rivaroxaban for VTE treatment.

Methods: Patients with objectively confirmed VTE, starting rivaroxaban and giving their informed consent were managed by the nurses and underwent a standardized questionnaire, a full educational training for treatment with rivaroxaban and were included in a registry. Demographic features and clinical history were checked. During follow-up patients were asked to contact us for treatment temporary or definitive interruption, dose reduction or bridging; all causes for changes were gathered. Monitoring of renal, hematologic and liver function every 4 weeks was planned for fragile patients.

Results: From June 1st to December 31st 2014, 55 patients were included. All patients answered the questionnaire. 4 patients had cancer, 4 patients had thrombophilia, no patients had renal insufficiency, 8 patients had haemodynamically stable PE. 5 patients completed their treatment course, 1 patient stopped treatment for a recurrence. 5 patients had minor bleeding, 1 had moderate renal insufficiency; all were reduced the dosage of rivaroxaban. 1 patient had major bleeding and treatment was temporarily interrupted. 4 patients asked informations for temporary interruption and 2 patients for bridging therapy. Patients were interviewed after a mean 3-month period and were fully satisfied of nursing assistance and of treatment with rivaroxaban.

Conclusion: Nursing management of VTE treatment with a full educational training, a standardized questionnaire, a registry and the direct contact with patients is an optimal tool to monitor the compliance of treatment with rivaroxaban

Disclosure of Interest: None declared.

PO386-MON

Response of leucocyte count, leucocyte differential count and thrombocyte count on frequent blood donations among male subjects in University of Calabar Teaching Hospital Donor Clinic, Calabar, Nigeria

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Background: Abuse of blood donation is common in Calabar, Nigeria. Most research on blood donors has been done only focusing on erythrocyte parameters without considering other cell line like leucocyte and thrombocyte of donors.

Aims: To assess the reaction of leucocyte and thrombocyte aside the erythrocyte parameters among frequent male blood donors in Calabar, Nigeria.

Methods: Materials: One hundred and eighty-four (184) subjects comprising five groups were recruited into this study, that is, 35 (19.0%) control group (donors donating for the first time), 32 (17.4%) of first (donors donating for the second time), 35 (19.0%) of second (donors donating for the third time), 41 (22.3%) of third (donors donating for the fourth time) and 41 (22.3%) of fourth (donors donating for the fifth time) time blood donors. Approval was from Health Research Ethical Committee (HREC) of UCTH and consent obtained. They were aged 18–49 years of age. First time blood donors of similar age were used as the control group. Their full blood counts were evaluated using complete automated cell counter (ERMA INC. Tokyo PCE-210, 5.10 version).

Results: Results showed that 93% of all donors were in the 18–35 age group and more than 63% of the donors were commercial blood donors. Hct of first, second, third and fourth time male blood donors were significantly reduced progressively as number of times of donation increased when compared with the control group. White blood cell (except for lymphocyte) and platelet counts also decreased progressively with repeated blood donation mostly among donors donating third and fourth time donors when compared with control group. Although, all the result gotten from leucocyte and thrombocyte were within the reference range in Africa.

Conclusion: Repeated (third and fourth timers) blood donation has shown in this study to produce a decrease in Hct, WBC (monocyte and granulocyte) and platelet counts when compared to the control while there was an increase in lymphocyte.

Disclosure of Interest: None declared.

PO387-MON

Participation of nurses in preparation of patients with hemophilia for surgery of colon cancer

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Background: Among the healthy people, control of bleeding is achieved very quickly and without any need for medical intervention. However, some people suffer from innate bleeding disorders that reduce their ability to achieve hemostasis. Many of these disorders are hereditary, such as hemophilia, which occurs due to complete or partial lack of factors that affect the normal blood coagulation. Adequate compensation of the missing clotting factor provides an implementation of more aggressive diagnostic, therapeutic, surgical-therapeutic procedures in patients with hemophilia in order to prevent bleeding after the intervention.

Aims: To identify activities of nurses in preoperative preparation of patients for internist surgical intervention.

Methods: **CASE STUDY:** Surgical intervention of colon cancer in patients with hemophilia

Results: Long-term prophylaxis is necessary in patients with hemophilia, and in the case of surgical intervention is obligatory. Given the proper prophylaxis and preparation of patients for surgical intervention, there is a need for 100% compensation of the missing factors to prevent bleeding during and after the intervention as well.

Conclusion: Number of patients with hemophilia is increasing which can be explained by advances in diagnosis and treatment. The quality and length of life of patients are improved. Today it is considered that with the adequate treatment of hemophilia patients can reach the length of life in the general population. Nurse technicians occupy an important place in the multidisciplinary work with people suffering from hemophilia and there is a need for their continuous education during their service life. Healthcare and educational work with patients is of crucial importance for patients and their families.

Disclosure of Interest: None declared.

PO388-MON

Mild haemophilia a: a manageable condition?

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Background: “Mild haemophilia is a very manageable condition. If you have mild haemophilia it may have little impact on your life as long as you know what to do and how to manage it” © HFA 2011

During the years I have practiced as a Clinical Nurse Specialist in Thrombosis/Haemostasis I have managed the cares of many patients with mild haemophilia. Many of these people only require treatment when they are having invasive procedures and individual care plans/surgical protocols are prepared for each patient.

Aims: To discuss three different cases of management of bleeding in elderly gentlemen with Mild Haemophilia A. Covering topics such as ageing complications and treatments, costs and safety issues for these patients.

Methods: Over the last few years we have treated three individual cases in men aged between 64–90 years and I would like to present their case histories and cover the complexity of treatment required for elderly people with mild haemophilia A.

Results: WM 64 year old man who required a Holmium Laser Enucleation of the prostate (HoLEP). This was indicated as a lower bleeding risk surgery and a protocol was prepared using Factor VIII to obtain levels of >80% with a gradual reduction over 7–10 days. JH 90 year old man who also required the above mentioned surgery and a similar protocol was prepared. SM 87 year old man who was admitted to hospital following a fall. His discharge was complicated by another fall which has had a very dramatic impact on his life and lifestyle. Each of these gentlemen experienced complications due not just to their bleeding condition but to other factors such as age related co-morbidities.

Conclusion: Even though many believe that Mild Haemophilia A is a fairly manageable condition occasionally complications arise and additional factors such as the ageing process can have adverse affects on the outcome for these patients. During this presentation/poster I would like to highlight the complexities we as nurses caring for this patient population need to be aware of.

Disclosure of Interest: None declared.

PO389-MON

Source data verification pilot: using central monitoring in an: observational critical care biomarker studyMcdonald E¹, Jain D¹, Ward R², Liaw P³ and Fox-Robichaud A¹¹Medicine, McMaster University, Hamilton; ²Research, Ottawa Hospital Research Institute, Ottawa; ³Research, Thrombosis and Atherosclerosis Research Institute TaARI, Hamilton, Canada

Background: Source Data Verification (SDV) is the process of ensuring that the collected data are accurate and reliable. The traditional gold-standard of 100% on-site monitoring results in large costs and logistical complexity leading to the development of novel central monitoring (CM) techniques. SDV has traditionally focused on clinical trials.

Aims: Our aim was to determine the feasibility of applying CM techniques to observational studies.

Methods: In this observational, retrospective SDV study we enrolled a convenience sample of 50 patients who were enrolled in The DYNAMICS Study.

DYNAMICS (DNA as a Prognostic Marker in ICU Patients) is a multi-centre observational study investigating plasma cell-free DNA as a biomarker for ICU mortality. We used source documents of patients that were previously sent to the Methods Centre for infection adjudication. We identified 9 clinically relevant data categories we felt were important for monitoring: Inclusion & Exclusion Criteria, Past Medical History, Sample Dates, Microbiological Cultures, ICU & hospital discharge status & date. The total sample was divided among 9 sites in proportion to recruitment. The electronic database data was compared with the original source documents.

Results: Of the 450 data points examined, 44 were missing from the source documents and excluded from the analysis. Each individual study site and data category had greater than 80% accurate data with the exception of ICU discharge/death date, which was 79%. Most notable was hospital and ICU final status data was 100% accurate. Overall, CM was found to be 93% accurate across 50 patients.

Conclusion: CM needs further development and research as SDV technique before it can be widely utilized in observational studies. Despite some limitations, this study has shown that CM techniques can be applied to observational studies and quality assurance research should not be constrained solely to 100% SDV in clinical trials, as has happened historically.

Disclosure of Interest: None declared.

PO390-MON

Raising awareness of venous thromboembolism prevention: how the Jewish general hospital is meeting the requirements for hospital accreditationEmed J^{1,2}, Strulovitch C³, Chung S² and Patel H²¹Ingram School of Nursing, McGill University; ²Nursing; ³Thrombosis, Jewish General Hospital, Montreal, Canada

Background: The Jewish General Hospital (JGH) (Montreal, Canada) Thrombosis Team has been engaged in activities aimed at providing information on venous thromboembolism (VTE) prevention and raising awareness about its importance to patient safety. In 2012, Accreditation Canada formalized the requirements for VTE prophylaxis by making it a condition for hospital accreditation. Several criteria are assessed: having a VTE prophylaxis policy, identifying patients at risk and providing appropriate prevention, ensuring post-discharge prophylaxis after major orthopedic surgery, measuring thromboprophylaxis performance and making improvements accordingly, and providing information to staff and patients about the risks and prevention of VTE.

Aims: To promote appropriate thromboprophylaxis through awareness-raising and educational activities for patients and staff.

Methods: During "March is DVT Awareness Month", several activities are organized at the JGH, including Medical Grand Rounds, Nursing Grand Rounds, an interactive test-your-knowledge "thrombolympiad" for residents and multidisciplinary team members, information kiosks, and a public lecture. A "super clotbuster" mascot also tours the hospital, answering questions and providing information. These presentations provide a forum for disseminating best practices in VTE prevention and sharing audit results, clinical issues and quality improvement efforts. Newsletter articles and poster displays act as communication tools to complement the campaign.

Results: At the last accreditation (2012), the JGH met the standards for VTE prevention, and the hospital was accredited with exemplary standing. The team is planning its 8th edition of DVT Awareness Month for 2015, which has become a well-established tradition at our hospital.

Conclusion: Awareness-raising activities can be an effective way of energizing and mobilizing staff and patients regarding VTE prevention. They are also a fun and creative way of meeting accreditation standards.

Disclosure of Interest: None declared.

PO391-MON

Haemophilia and ageing. Promoting healthy ageing through proactive engagement with general practitioners – literature review and pilot survey

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Background: Treatment advances in recent decades have increased life expectancy for men with haemophilia. These men are experiencing more general health concerns associated with advancing age. The management of age related conditions such as cardiovascular disease and cancer is costly and complicated in this population. In addition, these patients may not have as strong links to general practice as the wider population, with specialist haemophilia treatment centres in tertiary settings traditionally providing comprehensive care of the health needs of this population. It is unknown whether men are accessing recommended preventative health checks. This situation is exacerbated by reluctance of men to engage with health services, not knowing how to discuss the complexities of ageing and assumptions that men have about the knowledge of their general practitioner (GP) regarding haemophilia.

Aims: To assess the current status of preventative primary healthcare in the ageing haemophilia community.

Methods: A literature review was performed and a pilot survey developed to explore the current state of knowledge of men with haemophilia about general health and ageing, their level of engagement with general practitioners and involvement in general health screening. The survey was distributed to all men with haemophilia in Queensland, Australia, with results yet to be analysed.

Results: The outcome of the survey will inform further research, including focus groups where topics can be explored further and promoters and barriers to primary prevention of age-related conditions can be identified.

Conclusion: By engaging with men living with haemophilia in Queensland, Australia, this research aims to gain an initial understanding of the current dialogue between men with haemophilia and GPs around healthy ageing. Ultimately, online and paper resources will be developed to encourage and facilitate a proactive discussion about healthy ageing between men with haemophilia and their GP.

Disclosure of Interest: O. Hollingdrake Grant/Research Support from: Pfizer Nurses Education Award, B. Zeissink Grant/Research Support from: NovoNordisk "Changing Possibilities in Haemophilia".

PO392-MON

A survey of clinical research coordinators to manage the clinical research paperwork mountainSchnurr T¹, Cooke S², Kazimer D³, Sayles MJ³ and McDonald E⁴¹Thrombosis Research, St. Joseph's Healthcare Hamilton;²Medicine, HHSC; ³Research, SJHH; ⁴Medicine, McMaster University, Hamilton, Canada

Background: An essential component of the role of the Clinical Research Coordinator (CRC) is to ensure that all study required documentation is complete, accurate and accessible. The increasing scope, size, and complexity of clinical trials amplify the amount of paperwork required for each study. Fiscal constraints and staffing reductions increase the number of studies that individual CRCs are responsible for managing. The incongruity of the increasing need to process and track paper documents in a progressively electronic age represents a major challenge for CRCs.

Aims: In an attempt to explore current practice patterns and to share these practices to improve document management, we developed and distributed a survey to clinical research staff (excluding principal investigators) working at St. Joseph's Healthcare Hamilton (SJHH).

Methods: The survey used a mixed methods format, including demographic data about the CRC's area of expertise and years of experience, as well as qualitative questions to understand how CRCs manage the ever increasing complexity and volume of paperwork. Data was analyzed independently by three reviewers who then compared their findings to reach a consensus on general themes.

Results: The majority of CRCs at SJHH are women; most of whom have worked in the field of clinical trials (or of research) for 10 years or more. Colour coding and use of binders are the most common tools used to organize documents. Other organization strategies, tips and ideas were also identified.

Conclusion: Additional research is required to uncover the optimal strategies for managing the mountain of paperwork associated with managing clinical trials.

Disclosure of Interest: None declared.

PO393-MON

The Jewish general experience in developing patient teaching materials on warfarin and venous thromboembolismStrulovitch C¹, Emed J², Chung S² and Patel H²¹Thrombosis; ²Nursing, Jewish General Hospital, Montreal, Canada

Background: It is well-known that patient education on venous thromboembolism (VTE) and its treatment has a positive impact on our patient population. At the Jewish General Hospital (JGH), an informal survey with nursing staff revealed a need for patient education resources and many nurses requested increased information enabling them to adequately provide patient education. Importantly, the survey indicated a lack of available patient education resources.

Aims: To develop materials on VTE and anticoagulation (warfarin) to standardize and improve patient education.

Methods: A multidisciplinary committee (doctors, nurses, pharmacist, and dietician) was convened to review existing resources as a basis for creating our own printed materials. Once the contents of the booklets were completed, the booklets were reviewed by all members of our committee, by our hospital's Patient Education Network and a small pilot was conducted with a group of English-speaking patients to assess clarity and user-friendliness of contents. Work was also done with a translator and graphic designer to finalize the two booklets.

Results: Patient feedback from the pilot was positive, and the booklets were judged to be useful and informative. In-servicing was provided on all in-patient units (day and evening shifts) to review the booklets'

contents and the method of distribution and re-ordering. An auditing mechanism was introduced through the Anticoagulation Clinic to assess if nurses were giving the patients these booklets at time of discharge. It was found that there were inconsistencies in distribution amongst the different hospital units.

Conclusion: Booklets with key information on VTE and warfarin are deemed important and useful resources by patients and staff alike. Future plans are to ensure their optimal use and availability, make the content available online, and include new oral anticoagulants.

Disclosure of Interest: None declared.

Platelet activation / adhesion / aggregation I

PO394-MON

The impact of B3 cytoplasmic tail mimetic peptides on platelet aggregation and α IIb β 3 activationNguyen HTT^{1,2}, Huynh KC^{2,3}, Scharf RE^{1,4} and Stoldt VR^{1,2,4}¹Department of Experimental and Clinical Hemostasis, Hemotherapy, and Transfusion Medicine, Heinrich Heine University Medical Center; ²NRW Research School Biostruct, Heinrich Heine University, Dusseldorf, Germany; ³Department of Biomedical Engineering, International University - Vietnam National University, Ho Chi Minh City, Viet Nam; ⁴Biological Medical Research Center, Heinrich Heine University Medical Center, Dusseldorf, Germany

Background: The integrin-regulatory adaptor proteins talin-1 and kindlin-3 serve as important links between actin and integrins and are essential for their function.

Aims: This study aimed at exploring the effect of β 3 cytoplasmic tail mimetic peptides, disrupting the interaction between α IIb β 3 and integrin-regulatory adaptor proteins, on platelets aggregation and PAC-1 binding to platelets.

Methods: To study the effect of mimetic peptides on platelet aggregation, platelets (2×10^8 mL⁻¹) in platelet-rich plasma were incubated with mimetic peptides (800 μ M), containing the binding site for either talin-1 (PT) or kindlin-3 (PK). The peptides had been fused to a TAT sequence (⁴⁷YGRKKRRQRRR⁵⁷), which is known to facilitate the uptake of peptides into platelets. Subsequently, peptide-treated platelets were stimulated by 2.5 μ M ADP. Their response was recorded on an aggregometer. To examine the impact of peptides on α IIb β 3 activation, ADP-induced platelets, pretreated with mimetic peptides, were incubated with FITC-conjugated PAC-1. The level of PAC-1 binding was subsequently measured by flow cytometry. As control experiments, platelets without peptides or with control peptide (PC), containing the TAT sequence only, were used.

Results: Aggregation assays revealed that, in the presence of ADP, mimetic peptides caused a reduction by $15 \pm 3\%$ (PT) or $19 \pm 6\%$ (PK) in platelet aggregation, as compared to control experiments ($P < 0.01$, $n = 3$). FACS analysis showed that PT and PK resulted in a significant decrease by $16 \pm 5\%$ and $12 \pm 5\%$ in terms of PAC-1 binding to ADP-activated platelets, as compared to control experiments ($P < 0.05$, $n = 3$). Platelets penetrated with PC bound to PAC-1 antibody as similar as samples in the absence of peptides.

Conclusion: Our results indicate that β 3 cytoplasmic tail mimetic peptides for talin-1 or kindlin-3 binding sites can inhibit platelet aggregation and reduce activation of α IIb β 3 in response to agonists.

Disclosure of Interest: None declared.

PO395-MON

Bile salt chenodeoxycholate suppresses normal platelet functions through cyclic amp activation pathwayMetharom P¹, O'Gara F² and Berndt MC³¹Health Sciences; ²Curtin Health Innovation Research Institute;³Health Sciences, Curtin University, Perth, Australia

Background: Increased gastro-oesophageal reflux is common among patients with chronic obstructive pulmonary diseases. Studies indicate bile salts as one of the key effectors in aggravating bronchial complications. They are associated with persistent colonisation of pathogens found in cystic fibrosis and are known to exert both stimulatory and inhibitory effects in various cell types. Given that platelets circulate continuously through the pulmonary capillaries and participate in the maintenance of vascular integrity, it is possible that normal platelet functions are modulated by the presence of bile salts.

Aims: To investigate the impact of bile salts on platelet response to stimuli and the signalling pathways involved.

Methods: Platelet aggregometry, flow cytometry and western blotting.

Results: Primary tauro- and glyco-chenodeoxycholic acid (TCDCA and GCDCA), and secondary bile salts, lithocholic and deoxycholic acids, at 100 μ M, significantly suppressed washed platelet aggregation in response to collagen. In stimulated platelets, TCDCA inhibited Akt phosphorylation and increased the phosphorylation of cAMP-dependent PKA and VASP. Western blot and flow cytometry with different antibodies against TGR5, a bile receptor associated with cAMP elevation, confirmed positive TGR5 expression in platelets. Furthermore, a selective agonist of TGR5, INT777, inhibited platelet aggregation in response to collagen similarly to bile acid TCDCA.

Conclusion: Our data show for the first time that bile salts affect normal platelet function and signalling by increasing the activity of PKA and that the action is likely mediated through the receptor, TGR5. TGR5 agonists are in preclinical trial for treatment of various diseases including type-2 diabetes, atherosclerosis and obesity, all of which are associated with an increased risk of thrombosis. Our observations suggest that inhibition of platelet function by TGR5 activators might confer an additional cardiovascular benefit.

Disclosure of Interest: None declared.

PO396-MON

The role of heterotrimeric G proteins in regulation of GPIIb-IIIa activation and platelet contractilityBudnik I^{1,2}, Shenkman B³ and Savion N¹¹Goldschleger Eye Research Institute, Tel Aviv University, TelHashomer, Israel; ²Department of Pathophysiology, I. M.

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Russia; ³National Hemophilia Center, Sheba Medical Center, Tel Hashomer, Israel

Background: Most of soluble platelet agonists mediate their effect via receptors coupled to heterotrimeric G proteins.

Aims: To investigate the effect of sole and combined stimulation of G proteins on the activity state of α IIb β 3 and platelet contractility.

Methods: Blood was obtained from healthy volunteers who signed an informed consent form approved by the local research ethics committee. Activation of G α q, G α i and/or G α 12/13 proteins was achieved by using combinations of the following platelet agonists: ADP, epinephrine, arachidonic acid, U46619, and inhibitors: AR-C66096, MRS2500 and acetylsalicylic acid. Total expression and activity state of α IIb β 3 were evaluated by flow cytometry using P2 and PAC-1 mAbs, respectively. Platelet contractility was evaluated by modified rotational thromboelastometry in which heparinized platelet-rich plasma was pretreated or not with the platelet inhibitors indicated above, supple-

mented with reptilase and coagulation factor XIIIa and stimulated by platelet agonists.

Results: Selective stimulation of G α 12/13 or G α q did not cause α IIb β 3 activation. Selective stimulation of G α i and its combined stimulation with G α 12/13 or G α q proteins caused α IIb β 3 activation in the following order: G α i < G α q + G α i $\approx$ G α q + G α 12/13 < G α i + G α 12/13. Noteworthy, combined stimulation of G α i + G α 12/13 proteins produced greater effect than combined stimulation of all three G proteins. Combined stimulation of G α q + G α i + G α 12/13, G α q + G α i and G α i + G α 12/13 proteins as well as selective stimulation of G α i protein induced highly efficient platelet contractile response. In contrast, combined stimulation of G α q + G α 12/13 and selective stimulation of G α q or G α 12/13 proteins resulted in weak platelet contractility.

Conclusion: The results suggest an essential role for G α i protein in induction of maximal α IIb β 3 activation and platelet contractility and, therefore, in assembly of a functional fibrin- α IIb β 3-actin cytoskeleton complex.

Disclosure of Interest: None declared.

PO397-MON

The gut microbiota regulates von willebrand factor expression and platelet prothrombotic function via toll-like receptor-2

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Background: Innate immune signaling and thrombosis are closely intertwined processes. Recent reports have linked the gut microbiota and in particular its metabolic function to the development of atherosclerosis and cardiovascular diseases.

Aims: While Toll like receptor 2 (TLR2) signaling is involved in atherosclerosis development, contributions of TLR2 to thrombosis and the interdependence of microbial colonization and platelet and vessel wall function are currently unknown.

Methods: By the use of germfree (GF) mouse technology combined with intravital fluorescence microscopy we address if the microbiota could be a factor that fosters platelet adhesion.

Results: In static adhesion experiments we found reduced adhesion of platelets from *Tlr2*^{-/-} mice to collagen and laminin. To investigate whether intact TLR2 signaling is required for experimental thrombus formation, we visualized platelet deposition to the injured endothelium in a transient ligation model of the *Arteria carotis communis* using fluorescence intravital microscopy. Platelet deposition to the vessel wall was impaired in *Tlr2*^{-/-} mice, but no hemostatic abnormalities were detected. Platelet depletion experiments in mice and static adhesion experiments with washed platelets revealed that the adhesion defect in *Tlr2*^{-/-} mice was not platelet intrinsic, but was caused by a plasma-borne factor. *Tlr2*^{-/-} mice and thrombosis-protected GF mice showed reduced vWF plasma and liver mRNA levels compared with conventionally-raised wild-type (CONV-R) counterparts. GF wild-type and *Tlr2*^{-/-} mice were indistinguishable in plasma vWF levels and platelet deposition on injured arteries. In CONV-R *Tlr2*^{-/-} mice, the defect in platelet adhesion was rescued by addition of human vWF to levels seen in wild-type blood. In addition, adhesion of TLR2^{-/-} platelets to extracellular matrices was increased by supplementing with vWF.

Conclusion: Here, we report that microbiota-triggered TLR2 signaling in the liver supports vWF mediated platelet deposition in carotid ligation injury.

Disclosure of Interest: None declared.

PO398-MON

Nuclear receptor dependent formation of coated platelets inhibits platelet responses to stimuli

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Background: Liver X receptors (LXRs) and Farnesoid X receptor (FXR) have been identified in human platelets. Ligands for both have been shown to have non-genomic inhibitory effects on platelet activation, including inhibition of aggregation, granule secretion, adhesion and spreading. Interestingly, pathological conditions associated with increased circulating levels of LXR and FXR ligands have been related to platelet hyper-reactivity.

Aims: The role of these receptors in resting platelets was therefore determined.

Methods: Resting washed platelets were treated with increasing concentrations of LXR (Gw3965) and FxR (Gw4064) synthetic ligands and markers of platelet activation and procoagulant activity were determined in comparison to vehicle-treated platelets.

Results: Treatment with either Gw3965 or Gw4064 elicited a dose dependent increase in resting platelet activity, including increases in alpha granule secretion and fibrinogen binding. In addition Gw3965 and Gw4064 also elicited the formation of COATED platelets, as both caused an increase in phosphatidylserine exposure, increased intracellular calcium levels, depolarization of the mitochondrial membrane, generation of reactive oxygen species and formation of microparticles. This increase in activity was found to be dependent on intracellular calcium as treatment in the presence of BAPTA-AM prevented COATED platelet formation.

Conclusion: This and previous work suggest that activation of LXR and FXR converts a subset of platelets to the reactive procoagulant state. These platelets become desensitized and unable to respond to platelet stimuli resulting in inhibition of responses to classical platelet agonists. This has clinical implications since nuclear receptor ligands are under development for the treatment of several pathological conditions. Understanding these non-genomic effects of the nuclear receptors and their ligands is essential to balance their genomic regulatory roles against their possible additional effects on platelet reactivity and function.

Disclosure of Interest: None declared.

PO399-MON

Considerable variability of platelet response to tissue factor in healthy individuals and potential role of fxa inhibitor as an anti-platelet agent

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Background: In the view of physiological blood coagulation, the primary pathway leading to thrombus formation is the tissue factor (TF)-initiated coagulation pathway. During the initiation phase of coagulation, TF generates a small amount of thrombin. The small amount of thrombin is enough to activate platelets and then, platelet produces a burst of thrombin generation of sufficient magnitude to clot fibrinogen. Cardiovascular disease, such as acute coronary syndrome is associated with activation of platelets with higher thrombin generation levels. However the effect of anticoagulant agent to platelet activation is not fully considered.

Aims: The aim of this study is to evaluate the variability of platelet response to tissue factor in healthy individuals and the role of FXa inhibitor as a potential anti-platelet agent.

Methods: Human whole blood was collected from healthy volunteers in tubes containing 1/10 volume of 3.8% sodium citrate. Citrated human blood was centrifuged to obtain platelets-rich plasma (PRP). PRP response to TF was evaluated in vitro with expression of platelet-activation specific antigens using flow cytometric analysis.

Results: TF produced activation of human platelets within 2 min. TF induced platelet activation was observed in dose-dependent manner. However, considerable individual variability in platelets activation response to TF was observed. Platelets sensitivity varied over 20 folds in TF concentration. Inhibition of thrombin generation by blocking upstream proteases, FXa, by Rivaroxaban was as effective in preventing TF-induced platelet activation.

Conclusion: Considerable individual variability in platelets activation response to TF was observed. Blocking upstream coagulation factors higher than thrombin, which reduces thrombin production, could have the potential role as the anti-platelet agent.

Disclosure of Interest: None declared.

PO400-MON

Physiological regulation of platelet aggregation by the gasotransmitter hydrogen sulfide

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Background: Platelets are key drivers of acute cardiovascular events such as myocardial infarction. Hydrogen sulfide (H₂S) is a gasotransmitter that is enzymatically generated in biological tissues and with emerging roles in cardiovascular biology. There is currently no consensus on the regulation of platelets by H₂S.

Aims: We aimed to investigate the enzymatic source of H₂S in platelets and define its role in regulating platelets.

Methods: We assessed expression and catalytic activity of H₂S-generating enzymes in human platelets using Western blotting and enzymatic assays. We also determined the effect of a slow-releasing H₂S compound GYY4137 (morpholin-4-ium 4 methoxyphenyl(morpholino) phosphinodithioate) as well as inhibitors of H₂S generating enzymes upon human platelet aggregation *in vitro*. Additional studies were performed in a real-time mouse model of radiolabelled platelet thromboembolism.

Results: Western blotting showed that human platelets express the H₂S generating enzyme cystathionine- β -synthase (CBS) but not cystathionine- γ -lyase (CSE). In addition, CBS was catalytically active in platelets whereas CSE was not. Experiments with the H₂S selective probe WSP-1 showed that platelets generated H₂S and that H₂S production was inhibited by the CBS inhibitor 2-(aminoxy)acetic acid (1 mM). The slow releasing H₂S donor GYY4137 inhibited thrombin- and collagen-induced human platelet aggregation in a concentration-dependent manner and to a significantly larger extent than the decayed control compound. *In vivo*, GYY4137 (50 mg kg⁻¹) significantly reduced collagen-induced radiolabelled platelet aggregation.

Conclusion: Platelets generate H₂S endogenously from CBS. Furthermore, H₂S, when applied to platelets in a manner that mimics slow, physiological release from enzymes, inhibits platelet aggregation through mechanisms that remain to be identified. H₂S may therefore be an important physiological regulator of platelet activation.

Disclosure of Interest: None declared.

PO401-MON

Platelet-specific GARP deficiency does not affect thrombosis and hemostasis in mice

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Background: We previously identified Glycoprotein-A Repetitions Predominant protein (GARP or LRRC32) on human platelets and endothelial cells and provided evidence for its involvement in thrombus formation in GARP deficient zebrafish (Blood 2009; 113: 4754).

Aims: We now investigated the role of murine platelet GARP in thrombosis and hemostasis using platelet-specific conditional GARP knock out mice (GARP cKO).

Methods: Cre expression driven by the PF4 promoter was used to generate transgenic mice specifically lacking GARP expression on megakaryocytes and platelets. Hematological parameters (platelets, mean platelet volume, white blood cells, red blood cells, hemoglobin and hematocrit) and platelet surface glycoprotein expression (GPIb, GPIIb/IIIa and GPIIb) were determined in GARP cKO and littermate controls. Platelet activation was measured *ex vivo* by flow cytometry (P-selectin and α IIb β 3) and aggregometry (PAR4 and collagen) and *in vivo* by measuring the tail bleeding time and the occlusion time of the mesenteric and carotid artery after FeCl₃-induced thrombosis.

Results: No GARP could be detected on platelets of GARP cKO mice, in contrast to their controls, whereas an increased GARP expression was detected upon platelet activation. GARP cKO revealed unaltered hematological parameters and their platelets had normal surface glycoprotein expression. Furthermore, GARP cKO platelets displayed normal activation and aggregation responses compared to their controls. *In vivo*, GARP deficiency on platelets did not affect the tail bleeding time ($n = 10$) nor the occlusion time in the carotid ($n = 10$) or mesenteric artery ($n = 10$) thrombosis model.

Conclusion: These results provide evidence that murine platelet GARP is not required for a normal platelet function in thrombosis and hemostasis. Whether GARP on endothelial cells might influence thrombus formation is currently under investigation.

Disclosure of Interest: None declared.

PO402-MON

Construction of expression system for biologically functional recombinant rhodocytin

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Background: C-type lectin-like receptor 2 (CLEC-2) has been identified as a receptor for rhodocytin, a platelet activating snake venom from *Calloselasma rhodostoma*. Binding of rhodocytin to CLEC-2 initiates a signaling cascade leading to platelet activation and aggregation. Rhodocytin is secreted as a heterodimeric C-type lectin-like protein consisting of alpha- and beta- subunit with a disulfide bond. In general, snake venoms have been frequently used for research in thrombosis and hemostasis and have greatly contributed to the development of the research. Most of these venom proteins are purified from venom gland. Since the availability of snake venoms is limited, it is important to produce the recombinant snake venom proteins for research. It has not been reported the production of recombinant rhodocytin (rRhodocytin).

Aims: The aim of this study is to produce full-functional rRhodocytin with activity of platelet aggregation.

Methods: We constructed expression vectors containing reported gene sequence of rhodocytin that were optimized for expression in mamma-

lian cells. These expression vectors were transfected into CHO cells by electroporation. Medium and cell lysate were collected at 3 days after electroporation and analyzed for these recombinant proteins.

Results: Western blotting with anti-rhodocytin antibody showed that rRhodocytin was secreted to the medium only when alpha and beta-subunits were transfected together, suggesting that a disulfide bond is required for secretion. FACS analysis showed that rRhodocytin bound to 293 TREx cells expressing CLEC-2, but not their parental cells. rRhodocytin induced aggregation of human platelets and CLEC-2^{+/+} murine platelets, but not CLEC-2^{-/-} platelets.

Conclusion: We successfully generated the rRhodocytin that was able to aggregate platelets via CLEC-2. rRhodocytin can be used not only as a research tool but also as "a lead protein" in order to develop anti-CLEC-2 medicine, which is now under investigation.

Disclosure of Interest: None declared.

PO403-MON

Localization of Zn²⁺ in dense granules of human platelets and the extent of its release upon platelet activation

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Background: Zn²⁺ released from platelets modulates the contact pathway, fibrinolysis and other hemostatic processes. Platelet Ca²⁺ is stored in dense granules, and according to previous reports, Zn²⁺ is stored in alpha granules.

Aims: To clarify the site of Zn²⁺ storage in platelets and quantify release during activation.

Methods: Platelets were live-stained with combinations of FluoZin3-AM, MitoTracker deep red and calcium orange, fixed and stained with wheat germ agglutinin prior to imaging via high resolution fluorescence microscopy to determine subcellular localization of Zn²⁺, Ca²⁺ and mitochondria. To measure Zn²⁺ release, platelets were activated with thrombin and collagen or subjected to sonication, and Zn²⁺ and ADP in the releasates and lysates were quantified via atomic absorption spectroscopy and a fluorometric assay, respectively. Zn²⁺ content before and after activation was also quantified via flow cytometry.

Results: Imaging of resting platelets localized Zn²⁺ to 1-4 foci per platelet, while Ca²⁺ was localized to the dense granules and cytoplasm. Colocalization was only observed in dense granules. Zn²⁺ was not observed in the mitochondria, cytoplasm or the open canalicular system. Total and releasable Zn²⁺ in 10⁹ platelets mL⁻¹ were 27 ± 11 and 14 ± 1 μM respectively, corresponding to release of ~ 5 μM Zn²⁺ in blood. ADP concentration in releasate was 12 ± 8 μM, consistent with complete dense degranulation. Under flow cytometry the FluoZin3-AM fluorescence intensity was significantly ($P < 0.001$) reduced by 54 ± 2% after platelet activation, confirming that about half of platelet Zn²⁺ is releasable.

Conclusion: Zn²⁺ is packaged with Ca²⁺ in the dense granules of human platelets, and half the cellular content is releasable upon activation. Thus, platelet activation at sites of arterial injury has the potential to modulate hemostatic processes by substantially increasing local Zn²⁺ concentration.

Disclosure of Interest: None declared.

PO404-MON

Neutrophils contribute to the generation of procoagulant platelets by a myeloperoxidase independent mechanism

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Background: The procoagulant subset of highly activated platelets has been associated with increased pathological thrombotic states such as ischaemic stroke and sepsis. Increased generation of reactive oxygen species has been implicated in procoagulant platelet formation, thus we postulated a role for activated neutrophils and in particular neutrophil myeloperoxidase (MPO) in procoagulant platelet associated occlusive thrombosis. We have shown a novel marker for procoagulant platelets, trivalent arsenical GSAO, identifies procoagulant, necrotic platelets in a whole blood flow cytometry assay and enables direct visualisation of fibrin supporting platelets in occlusive thrombi after 8% FeCl₃ injury.

Aims: We used GSAO to study the role of activated neutrophils and MPO in generation of procoagulant platelets in vitro and in vivo.

Methods: Thrombus formation in the 8% FeCl₃ model of thrombosis in the murine cremasteric arterial circulation was visualised using high speed confocal intravital microscopy in presence of anti-fibrin, anti-CD42b and GSAO. Neutrophil depletion was achieved by 48 h exposure to 1A8 anti-Ly6G.

Results: Compared with controls, neutrophil-depleted mice demonstrated decreased GSAO+ platelets, decreased occlusive thrombi and reduction in fibrin formation indicating a role for neutrophils in formation of procoagulant platelets in vivo. We examined whether the mechanism for this neutrophil effect involved MPO and downstream oxidants. However, ex-vivo addition of HOCl to washed human platelets did not induce formation of procoagulant platelets. We further investigated murine models of altered MPO levels and found no difference in procoagulant platelet formation in MPO^{-/-} mice versus WT C57Bl6 controls; and no difference in mice exposed to increased circulating MPO via 30 day continuous infusion compared with albumin control mice.

Conclusion: Thus, neutrophils are important for the generation of procoagulant, fibrin supporting platelets in vivo. However, the mechanism is not via MPO.

Disclosure of Interest: None declared.

PO405-MON

Impaired response to collagen of platelets from hyperlipidemic mice

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Background: Platelet function is modulated by lipoproteins. Correspondingly, we showed that elevated plasma HDL-cholesterol due to HDL receptor dysfunction increases platelet cholesterol content which affects platelet count and reactivity in mice and humans.

Aims: The aim of this study was to investigate platelet responsiveness under conditions of elevated plasma (V)LDL-cholesterol.

Methods: Experiments were performed with platelets from apolipoprotein E-deficient (apoE KO) mice, with elevated plasma (V)LDL-cholesterol (total cholesterol (TC) 396 ± 67 mg dL⁻¹), their wildtype (WT) littermates (32 ± 13 mg dL⁻¹), or apoE KO mice transplanted with WT bone marrow (BM) (46 ± 9 mg dL⁻¹).

Results: ApoE deficiency significantly reduced platelet counts. Bone marrow histology did not show a difference in megakaryocyte number, indicating that the reduced platelet count may be caused by increased clearance. Platelets from apoE KO mice contained 5.7-fold more cholesterol than wild-type platelets ($P < 0.001$). While the response to PAR-4 peptide was unaltered, activation of integrin $\alpha_{IIb}\beta_3$ in response to CRP-XL was significantly reduced for platelets from apoE KO mice (-32% vs. WT, $P < 0.05$). Similarly, the amount of surface-expressed P-selectin was 5.7-fold lower after CRP-XL stimulation ($P < 0.05$). Normalization of plasma TC by reintroducing apoE KO in hematopoietic cells by WT bone marrow transplantation restored CRP-XL-induced $\alpha_{IIb}\beta_3$ activation ($+126\%$, $P < 0.05$ vs. apoE KO BM-transplanted mice). The increased cholesterol content of apoE KO platelets may be responsible for the observed differences, by changing lipid rafts to which the collagen receptor GPVI localizes after activation, or by activating the cellular cholesterol sensor LXR, which affects GPVI signaling.

Conclusion: *In vivo*, collagen, exposed after vessel wall injury, activates platelets. Our findings may reflect a protective mechanism to prevent early platelet activation upon vessel wall injury under hyperlipidemic conditions.

Disclosure of Interest: None declared.

PO406-MON

Improving the quantification of in vitro thrombus formation

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Background: The diversity of flow systems and quantification methods make comparisons between in vitro thrombus formation experiments difficult. Several parameters can be used for quantification, e.g. surface coverage, fluorescent intensity or thrombus volume. However, these parameters may not truly reflect the actual accumulation of platelets since thrombus volume and coverage measurements does not separate thrombus increment by platelet accumulation from the volume reducing parallel process of platelet contractility, and fluorescence intensity measured in a single focal plane at the surface may lead to underestimation of aggregating platelets further out compared to adhering platelets at the surface. These issues might generate a misrepresentative result and the need for standardization in this area is high.

Aims: We aimed to construct a robust method for analysing thrombus formation, providing information on thrombus volume and density of platelets in the thrombus, thereby also information about platelet contractility.

Methods: Hirudin whole blood was drawn through a 200 μ m wide flow channel with a collagen patch at a wall shear rate of 1400 s⁻¹. The platelets in 5% of the blood volume were fluorescently labelled and micrographs were captured using time-lapse and z-stack. A MATLAB-script was used for image analysis and extraction of platelet number and position in the thrombus.

Results: Labelled platelets were detected and the mean distance to closest neighbours was determined. From this information we calculated the total number of platelets and estimated the volume of the thrombus. The density of platelets in the thrombus was calculated, yielding information about the platelet contractility. The volume estimation was verified with confocal microscopy.

Conclusion: The combined information of platelet number, thrombus volume and platelet density in the thrombus can give important addi-

tional information regarding thrombus development and lead towards better standardization.

Disclosure of Interest: None declared.

PO407-MON

RS6929846-dependent regulation of *btn2a1* expression in innate and adaptive immune cells affects platelet reactivity to thrombin

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Background: Platelet reactivity varies between individuals and this may in part explain differences in susceptibility to arterial thrombosis and the associated life-threatening conditions: stroke and myocardial infarction (MI).

Aims: We performed a genome-wide association study (GWAS) to identify genetic variants that may account for part of the corresponding variation.

Methods: We measured platelet reactivity for 268 healthy individuals of Northern European descent following ex vivo stimulation with ADP, collagen, and thrombin receptor activating peptide (SFLLRN). All individuals had been genotyped for > 700K SNPs, and transcriptome analysis performed for platelets, CD19 + B lymphocytes, CD4 + T helper lymphocytes, CD8 + T cytotoxic lymphocytes, CD14 + monocytes, and CD15 + neutrophils.

Results: We identified three regions exceeding the genome-wide significance level, respectively in 2q22.3 (collagen), 6p22.2 (SFLLRN), and 13q21.31 (collagen). None of the top SNPs were coding variants suggesting regulatory effects. The lead SNP for the 6p22.2 signal (rs6929846), located within the 5'UTR of the *BTN2A1* gene, has previously been associated with several inflammation-related pathologies including MI. We observed a cis-eQTL with *P*-value < 10⁻⁵ in CD8, and CD15 for *BTN2A1*. The association patterns for SFLLRN-dependent platelet reactivity and *BTN2A1* expression were highly correlated (*r*~0.9) in the two cell types, strongly suggesting that they are causally related. To our surprise there was no evidence for a *BTN2A1* cis-eQTL signal in platelets, hence suggesting an indirect effect. The rs6929846 T allele was associated with decreased reactivity to SFLLRN as well as with a higher basal level of platelet activation (*P* = 0.03).

Conclusion: The rs6929846 T allele may affect platelet activation by regulating the expression of *BTN2A1* in T helper lymphocytes and neutrophils, which may be linked to its demonstrated association with a pro-inflammatory status and MI.

Disclosure of Interest: None declared.

PO408-MON

Mouse CLEC-2 can support platelet adhesion to mouse podoplanin under arterial flow

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Background: The C-type lectin receptor CLEC-2 is highly expressed on the surface of platelets. The CLEC-2 ligand, Podoplanin, is expressed on a wide range of cell types including lymphatic endothelial cells but is not present in the blood vasculature. Podoplanin is also upregulated during inflammation, including on macrophages and Th17 cells.

Recently, we have shown that human Podoplanin supports adhesion of mouse and human platelets at venous rates of shear. In the present study we demonstrate the unexpected observation that mouse Podoplanin also supports adhesion and aggregation of mouse platelets at arterial rates of flow.

Aims: To investigate platelet adhesion to Podoplanin at varying shear rates.

Methods: Wild type and CLEC-2-deficient mouse blood was flown over capillaries coated with recombinant mouse Podoplanin at a range of shear rates. The effect of various inhibitors on platelet adhesion and aggregation was investigated.

Results: Platelets from mouse blood adhered to recombinant mouse Podoplanin at low shear rates (100–400 s⁻¹) and formed aggregates at higher rates of shear (500 - 1500 s⁻¹). Adhesion and aggregation were abolished using CLEC-2-deficient blood. Aggregation was markedly reduced in the presence of the Src kinase inhibitor Dasatinib, blockers of integrin α IIb β 3 and GpIb-IX-V, and inhibitors of ADP and thromboxane A2 (Cangrelor and indomethacin, respectively). The pathophysiological significance of platelet adhesion and aggregation at high shear was investigated in an ApoE^{-/-} mouse model. The CLEC-2^{-/-}, ApoE^{-/-} mice had a significantly higher plaque burden compared to ApoE^{-/-} on high fat diet.

Conclusion: This study demonstrates the novel observation that Podoplanin can mediate platelet adhesion and aggregation at arteriolar rates of shear. This may have pathological significance at sites of Podoplanin upregulation during vascular inflammation.

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Disclosure of Interest: None declared.

PO409-MON

AKT translocation and phosphorylation regulates platelet aggregation downstream of G12/13 and Gi pathways

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Background: Platelet agonists activate platelets directly or indirectly through G protein coupled receptors (GPCR) coupled to different G proteins, including Gq, Gi, and G12/13. Previously, two groups found that coactivation of Gi and G12/13 signaling pathway is sufficient to induce platelet aggregation. We found that this is true only at high concentration of agonists.

Aims: To further study platelet aggregation downstream of coactivation of G12/13 and Gi and the underlying mechanism.

Methods: Platelet aggregation was assayed using aspirin-treated washed platelets from human and mice stimulated with different concentration of 2-MeSADP plus AYPGKF in the presence of Gq inhibitor YM-254890. GTP-bound RhoA and intracellular cAMP were measured to confirm G12/13 and Gi activation, respectively.

Results: Costimulation of G12/13 and Gi pathways only induces platelet aggregation under higher concentration of agonists. Low concentration of 2-MeSADP plus AYPGKF does not induce platelet aggregation, though there is simultaneous G12/13 and Gi pathways activation as evidenced by dramatic cAMP decrease and RhoA activation similar to that induced by high concentration of agonists. Total Akt phosphorylation (Ser473 and Thr308) was observed at both low and high concentrations of agonists with high concentration elicited more robust pAkt. Akt translocation was also significantly higher in the presence of high concentration of agonists compared with the low concentration. Impressively, membrane Akt phosphorylation was not observed at low concentration of agonists in contrast to the robust membrane Akt phosphorylation caused by high concentration. Finally, we found that PI3k/PAK/Akt pathway mediates platelet aggregation downstream of Gi and G12/13.

Conclusion: Platelet aggregation downstream of coactivation of G12/13 and Gi pathways depends on agonist concentrations. Membrane translocated Akt phosphorylation is the convergence point regulating platelet aggregation downstream of Gi and G12/13 via PI3k/PAK/Akt pathway.

Disclosure of Interest: None declared.

PO410-MON

Effects of cyclic nucleotides on platelet aggregation and biological response modifier release induced by *Streptococcus sanguinis* strain 2017-78

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Background: Previous studies have shown that *Streptococcus sanguinis* (*S. sanguinis*) strain 2017-78 causes both the aggregation of, and secretion of biological response modifiers (BRMs) from, human platelets. Epinephrine significantly enhances, in a dose-dependent manner, *S. sanguinis*-induced aggregation, but paradoxically inhibits the release of the BRMs, sCD40L, PF4 and PDGF. In addition, epinephrine has been reported to inhibit adenylyl cyclase and stimulate guanylate cyclase activity in platelets.

Aims: The aims of the current study were to determine the stimulation of adenylyl cyclase and guanylate cyclase on *S. sanguinis* -induced platelet activation, and to examine any relationship between adenylyl cyclase and guanylate cyclase on the effects of epinephrine on *S. sanguinis* -induced platelet activation.

Methods: Aggregation of human platelets was determined by increased light transmission of platelet rich plasma and the release of the BRMs was quantified by ELISA.

Results: Pre-treatment of platelets with the selective activator of adenylyl cyclase prostacyclin (PGI₂; (0.1–1 µM) inhibited *S. sanguinis* strain 2017-78-induced aggregation and this was accompanied by an inhibition of secretion of sCD40L, PF4 and PDGF, with complete secretion completely abolished by 1 nM PGI₂. The selective activator of guanylyl cyclase sodium nitroprusside (NaNP) inhibited platelet aggregation and the secretion PDGF, PF4 and sCD40L in response to *S. sanguinis* strain 2017-78, with aggregation and secretion all completely abolished by 1 µM NaNP. The inhibitory action of epinephrine on strain 2017-78-induced release of PDGF, PF4 and sCD40L was unaffected by pre-treatment with either PGI₂ or NaNP.

Conclusion: As epinephrine, which decreases adenylyl cyclase activity, and PGI₂, which increases adenylyl cyclase activity, both attenuate the release of BRMs, it is unlikely that adenylyl cyclase is responsible for the control of BRM release.

Disclosure of Interest: None declared.

PO411-MON

Combined roles of factor XIIIa and glycoprotein IIB/IIIA in platelet-driven fibrin formation

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Background: Functional studies suggest roles for coated platelets in the local assembly of α-granular proteins and the stimulation of thrombin

generation. Yet, whether the high fibrin(ogen) binding on coated platelets is of functional significance is unclear.

Aims: We investigated the roles of the transglutaminase factor XIIIa (FXIIIa), and GPIIb/IIIa in coated platelet formation and function.

Methods: Washed human platelets were stimulated with combinations of thrombin and the collagen receptor agonist convulxin. Platelets in plasma were activated with tissue factor plus CaCl₂. Subpopulations of coated platelets were identified by dual-color flow cytometry, using fluorescent fibrinogen and a transglutaminase-dependent probe, A14 α₂-antiplasmin (A14-αAP). Whole blood from FXIIIa-deficient mice was used for flow perfusion studies.

Results: Stimulation of washed platelets with convulxin/thrombin resulted in a platelet population binding annexin A5 (indicative for PS exposure), factor Va and factor Xa. This population persistently showed high fibrinogen binding, but gradually decreased PAC-1 mAb binding, indicating slow inactivation of GPIIb/IIIa. In addition, they stained positively for fibrin and time-dependently incorporated A14-αAP in a FXIIIa-dependent way. Markedly, the high fibrin(ogen) binding was only antagonized by combined inhibition of GPIIb/IIIa and transglutaminase activity. In tissue factor-triggered platelet-rich plasma, high (fibrin)ogen binding was dependent on GPIIb/IIIa and FXIII. In whole blood perfusion, platelet-driven fibrin formation was abolished by GPIIb/IIIa blockage in FXIIIa-deficient mice. In congenital FXIII-deficient plasma fibrin formation on the platelet surface under flow was unchanged due to rescue by platelet-released FXIII.

Conclusion: Coated platelets, with initial GPIIb/IIIa activation and subsequent transglutaminase activity, collect multiple coagulation proteins and nucleate fibrin fiber formation in a FXIIIa-dependent way.

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PO412-MON

Pivotal role of SGK1 in the regulation of platelet secretory proteome, dense granule biogenesis and secretion

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Background: Platelet secretion is critical to the development of acute thrombotic occlusion. Platelet dense granules contain a variety of important hemostatically active substances. Nevertheless, biogenesis of platelet granules is poorly understood. SGK1 has been shown to be highly expressed in platelets and megakaryocytes, but its role in the regulation of platelet granule biogenesis and its impact on thrombosis has not been investigated so far.

Aims: The present study explored the role of SGK1 in the regulation of platelet secretory proteome, dense granule biogenesis and secretion and its impact on thrombus formation *in vivo*.

Methods: Electron microscopy; Proteomics; ATP-release; FACS and PCR analysis; *in vivo* adhesion and thrombus formation.

Results: Analysis of platelet ultrastructure revealed significant reduction in the number and packaging of dense granules in platelets lacking SGK1 (*sgk1^{-/-}*). In *sgk1^{-/-}* platelets serotonin content was significantly reduced and activation-dependent secretion of ATP, serotonin and CD63 was significantly impaired. *In vivo* adhesion of platelets was significantly decreased in platelets lacking SGK1 and *in vivo* occlusive thrombus formation was significantly diminished in *sgk1^{-/-}* mice. Proteomic analysis identified 10 vesicular transport proteins, including Rab27b, regulated by SGK1 which were significantly downregulated in *sgk1^{-/-}* platelets. Transcript levels and protein abundance of Rab27b were significantly reduced in *sgk1^{-/-}* platelets without affecting Rab27b mRNA stability. *Sgk1^{-/-}* megakaryocytes showed significantly reduced expression of Rab27b and serotonin/CD63 levels compared to *sgk1^{+/+}* megakaryocytes.

Conclusion: The present observations unraveled SGK1 as a novel powerful regulator of several platelet vesicular transport proteins affecting platelet dense granule biogenesis, platelet secretion and thrombus formation. SGK1 exerts its effects at least partially by regulating transcription of Rab27b in megakaryocytes.

Disclosure of Interest: None declared.

PO413-MON

Impact of elevated shear stress on integrin α IIb β 3 – ligand interaction

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Background: Shear stress can activate platelets leading to increased platelet aggregation in association with diverse vascular diseases. α IIb β 3-mediated signaling is directly involved in this process. We examined how shear stress effects the α IIb β 3–ligand interaction detecting the signaling of Src and FAK tyrosine kinases in fibrinogen-adherent human platelets.

Aims: We compared Src and FAK signaling between fibrinogen-adherent platelets and platelets on BSA matrix in response to shear stress with and without the α IIb β 3-antagonist abciximab.

Methods: Adhesion assays were performed on a cone-plate viscometer with 100 μ g mL⁻¹ of immobilized fibrinogen or 1% BSA using washed human platelets. Specific phosphorylation of Src (Y418) and FAK (Y397) were determined by Western blotting.

Results: In response to abnormal shear rates (5000 s⁻¹), platelets adherent onto fibrinogen exhibited 3-fold higher activities of Src and FAK signaling after 2 min incubation time than platelets under static conditions ($P < 0.05$). No such effect was seen at a shear rate of 500 s⁻¹. Shear stress alone induced platelet signaling with considerably higher kinase activities at a shear rate of 5000 s⁻¹ (10-fold increase, $P < 0.05$) compared to 500 s⁻¹. While Src activation in platelets exposed to 5000 s⁻¹ over BSA was about half as high as that in platelets exposed to 5000 s⁻¹ on immobilized fibrinogen, FAK signaling in platelets was similar under both conditions. Incubation with abciximab inhibited Src and FAK signaling in platelets at 5000 s⁻¹ both on fibrinogen and BSA ($P < 0.05$).

Conclusion: Abnormally high shear stress significantly increases platelet signaling compared to platelets under static conditions. While Src activation remains ligand-dependent in fibrinogen-adherent platelets even under shear stress, FAK signalling can be directly induced by shear. Abciximab significantly inhibits signaling even in the absence of fibrinogen indicating an important role of α IIb β 3 in shear-induced platelet activation.

Disclosure of Interest: None declared.

PO414-MON

Platelet function is regulated by acetylation and deacetylation mechanisms controlled by sirtuins

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Background: Protein acetylation is regulated by the activity of acetyl transferases and deacetylases (including sirtuins). The role of sirtuins on platelet function is unknown.

Aims: To explore the presence and role of sirtuins on platelet function.

Methods: We used washed platelets of healthy subjects who had not been given any medication. Inhibitors of sirtuins (sirt): cambinol (sirt1 and sirt2 inhibitor), AGK2 (specific of sirt2), sirtinol (specific of sirt1), and trichostatin A (inhibitor of non-sirtuin deacetylases). Platelets were stimulated with collagen, thrombin or U46619. Aggregation was studied by optical aggregometry, dense granule release (¹⁴C-serotonin), level of cytosolic calcium in platelets labeled with FURA 2AM, and acetylation or phosphorylation of proteins by immunoblotting using specific antibodies.

Results: Inhibition of sirtuins with cambinol resulted in a remarkable reduction of platelet responses (aggregation, granule release and cytosolic calcium ($P < 0.05$)). Inhibition of sirt2 produced a similar inhibition of platelet function as cambinol, while the inhibition of sirt1 or the non-sirtuin deacetylases had scarce effect. The presence of sirt2 in platelets, but not of sirt1, was demonstrated by immunodetection and confirmed by the presence of mRNA. Inhibition of sirt2 increased the acetylation of Akt, which in turn resulted in the blocking of agonist-induced Akt phosphorylation, a marker of its activity. In contrast, erk1/2 acetylation was not affected by treatments. Aggregation induced time-dependent changes in the acetylation pattern of proteins and increased sirt2 phosphorylation, a regulatory mechanism of sirt2 activity, as well as a time-dependent deacetylation of Akt.

Conclusion: The study reveals the presence of sirt2 in platelets and the central role of acetylation of proteins regulated by sirt2 on platelet function.

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PO415-MON

Fibrin stimulates spreading and activation of platelets through GPVI

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Background: Fibrin is formed from soluble fibrinogen by the action of thrombin. As the final product of the coagulation cascade, it plays a crucial role in the formation of stable thrombi. Glycoprotein (GP) VI, the major collagen receptor, has been shown to play a role in embolization of thrombi, which is paradoxical as collagen is only found at the base of a thrombus.

Aims: We hypothesise that GPVI may be a novel fibrin receptor.

Methods: Protein tyrosine phosphorylation, and spreading of human and mouse platelets was investigated using established techniques.

Results: Thrombin stimulated a marked increase in tyrosine phosphorylation in washed human platelets that were allowed to aggregate. Tyrosine phosphorylation was reduced in the presence of the α IIb β 3 blocker, eptifibatide, and blocked in the presence of GPRP (single amino acid

code), which blocks fibrin formation. Immunoprecipitation and Western blotting studies identified Syk and the FcR g-chain as major phosphorylated proteins. Similar results were observed in washed mouse platelets. An increase in tyrosine phosphorylation including in response to thrombin was also observed in GPIIb-deficient mice platelets which was also blocked by GPRP. This increase was absent in GPVI-FcR g-chain-deficient mouse platelets. Moreover, platelets spread on fibrin-coated surfaces to a greater extent than those on fibrinogen-coated surfaces. This was not the case for GPVI-deficient platelets or in the presence of a Src kinase inhibitor.

Conclusion: These results show that fibrin activates GPVI independently of α IIB β 3. This may explain the increase in embolization in the absence of GPVI at arterial rates of shear.

Disclosure of Interest: None declared.

PO416-MON

KINDLIN-2 in megakaryocyte and platelet functions

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Background: Kindlins (1, 2 and 3) play crucial roles in integrin activation processes by directly interacting with β -subunits. In platelets and megakaryocytes (MKs), kindlin-3 (K3) is the major form present, kindlin-1 has never been detected and kindlin-2 (K2) has been reported in platelets.

Aims: Our goal was to study K2 expression and function in MKs and platelets.

Methods: Kindlins' localization in MKs and platelets were performed using confocal and total internal reflection fluorescence microscopy. Lentiviral transduction allowed us to study K2 overexpression in CMK cells. Kindlins' phosphorylation was studied after immune-precipitation followed by western-blot analysis.

Results: In humans, we confirmed K2 expression in platelets and showed its presence in bone marrow resident megakaryocytes. We further demonstrated that, as K3, K2 expression increases during human megakaryocytic cell lines (HEL, Meg-01 and CMK) differentiation. In fibrinogen adhering CMK, K2 co-localizes with β_3 -integrins and is grouped in specific areas at the tip of F-actin fibers (focal adhesion). This K2 clusters are not observed in collagen-adhering CMK. Furthermore, K3 is absent from the F-actin fiber tips, the focal adhesion and does not co-localize with K2. These data suggest distinct roles for K2 and K3 in the α IIB β 3 integrin adhesion and spreading processes. K2 overexpression in differentiated CMK leads to significant decrease in adhesion and spreading over immobilized fibrinogen as well as collagen and fibronectin. Furthermore, reduced CMK aggregation in response to PMA was noticed upon K2 overexpression.

In fibrinogen-adhering platelets, K2 localizes along the F-actin fibers within lamellipodia. Upon platelet activation and adhesion to fibrinogen, not only K3 but also K2 is phosphorylated on serine/threonine and tyrosine residues respectively.

Conclusion: Altogether, these results indicate participation of K2 in both outside-in and inside-out integrin signaling pathways.

Disclosure of Interest: None declared.

PO417-MON

THE PRO33 (HPA-1B) isoform of integrin α IIB β 3 is indeed a prothrombotic receptor variant

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Background: The HPA-1 polymorphism of the β_3 gene of α IIB β 3 arises from a leucine $\rightarrow$ proline exchange at residue 33 of the mature β_3 subunit, resulting in HPA-1a (Leu33) or HPA-1b (Pro33) platelets. Using a case-only design in patients with coronary artery disease, we have documented that carriers of HPA-1b experience their myocardial infarction 5.2 years (median) earlier than HPA-1a/1a patients.

Aims: To explore the genotype-phenotype relation, functional and biochemical properties of HPA-1b (Pro33) platelets were studied in vitro.

Methods: Shear-induced cell adhesion onto immobilized fibrinogen was examined by digital imaging of fluorescently tagged platelets or transfected CHO cells expressing either isoform of α IIB β 3, using an established model simulating arterial flow conditions. Specific phosphorylation of Src was determined by Western blotting and quantified by densitometry.

Results: At a shear rate of 1500 sec⁻¹, adhesion activity (in PPACK blood) and thrombus formation (in citrated blood) of Pro33 platelets was increased 2.5-fold compared with homozygous Leu33 platelets ($P < 0.01$). Analysis of Src, a tyrosine kinase constitutively associated with α IIB β 3, revealed that activation of the phosphotyrosine motif at residue 418 was 4-fold higher in adherent Pro33 than Leu33 platelets ($P < 0.01$). Likewise, in transfected CHO cell clones expressing either isoform of α IIB β 3, adherent Pro33 cells exhibited a 3-fold higher level of Src pY418 than Leu33 cells ($P < 0.05$). Upon exposure of adherent α IIB β 3-CHO cells to stepwise increasing shear rates (up to 1500 sec⁻¹) over 10 min, the Pro33 variant displayed a higher adhesion stability than the Leu33 isoform. The rate of residual adherent cells was $82 \pm 5\%$ with Pro33 and $25 \pm 4\%$ with Leu33 cells, as compared to baseline (100%; $P < 0.0001$).

Conclusion: Pro33 platelets are characterized by increased adhesion activity, increased thrombus stability, and increased outside-in signaling corresponding to their prothrombotic phenotype.

Disclosure of Interest: None declared.

PO418-MON

The PRO33 variant of platelet α IIb β 3: allosteric changes resulting from a point mutation in the integrin ectodomain are associated with a prothrombotic receptor phenotype

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Background: The Pro33 variant (also designated HPA-1b) of platelet integrin α IIB β 3 arises from a proline $\rightarrow$ leucine exchange at residue 33 of the β_3 subunit and can have prothrombotic properties. Thus, Pro33 platelets display increased adhesion, increased thrombus stability, and increased outside-in signaling.

Aims: To explore the nature of this phenotype and underlying mechanisms at an atomic level, we have now 1) generated a model expressing fluorescent proteins fused with α IIB β 3 in transfected cells and 2) performed molecular dynamics simulations of the integrin ectodomain.

Methods: Transfected and fluorescently tagged HEK293 cells stably expressing either Leu33 or Pro33 of α IIB β 3 were generated. Cyan or yellow fluorescent proteins (CFP; YFP) were cloned to the C-termini of the α IIB or β 3 subunit prior to transfection. To explore conformational changes in the cytoplasmic tails upon integrin activation, measurements by FRET were performed. Molecular dynamics simulations in explicit solvent of in total 1 μ s length were applied to study consequences of the Pro33Leu exchange on structure and dynamics of α IIB β 3.

Results: Upon activation by outside-in signaling, FRET analyses indicated a more extended spatial separation (> 100 Å) of the cytoplasmic tails (α IIB-CFP; β 3-YFP) in Pro33 than in Leu33 cell clones ($P = 0.003$). Molecular dynamics simulations revealed that the Pro33 isoform of α IIB β 3 is, in line with its phenotypic properties, more easily activatable, as compared to Leu33. In atomic detail, the simulations indicated that the Pro33 variant results in a loss of stabilizing interactions of the PSI domain in the head region with the nearby EGF-I and EGF-II domains located in the leg region of α IIB β 3, allowing the protein to open and become active more rapidly.

Conclusion: These results provide insights on how a point mutation at a position > 90 Å away from any binding site in α IIB β 3 can allosterically affect the fine-tuned structural dynamics of the integrin.

Disclosure of Interest: None declared.

PO419-MON

Vascular smooth muscle cells stimulate platelets via CLEC-2: involvement of intracellular and extracellular ligands of CLEC-2

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Background: At the site of plaque erosion and late stent thrombosis, vascular smooth muscle cells (VSMCs) are supposed to be exposed to blood flow.

Aims: To investigate whether VSMCs activates platelets and if this is the case, its mechanism.

Methods: Described in the Result.

Results: Cultured VSMCs did not induce platelet aggregation, but granule release. Thrombus formation was observed when whole blood flowed onto immobilized VSMCs. These VSMC-induced platelet activation were significantly inhibited in C-type lectin-like receptor 2 (CLEC-2)-deficient mice, suggesting that VSMCs activates platelets via CLEC-2. Binding of recombinant CLEC-2 (rCLEC-2) to VSMCs was confirmed by flowcytometry and immunocytochemistry, but VSMCs did not express podoplanin, an endogenous ligand of CLEC-2. Protein array detected S100A13 as a rCLEC-2 binding protein. Biacore showed that S100A13 dose-dependently bound to rCLEC-2. Adhesion of CLEC-2-deficient platelets to immobilized S100A13 was significantly inhibited, suggesting that S100A13 binds to CLEC-2. S100A13 did not induce platelet aggregation, but immobilized S100A13 potentiated thrombus formation on the collagen-coated surfaces under flow. However, flowcytometry revealed that S100A13 was not expressed on the surface of intact VSMCs, although intracellular expression was confirmed by Western blotting, suggesting that there is other ligand of CLEC-2 responsible for VSMCs-induced platelet activation. Since S100A13 is known to be expressed on damaged cell surface, we examined the effect of the oxidative stress with H₂O₂ on VSMCs. Pretreatment of VSMCs with H₂O₂ induced surface expression of S100A13. Histological analysis showed that S100A13 was expressed in the lumen area, which is exposed to blood flow, in the atherosclerotic vessel wall from ApoE-deficient mice, but not in the normal vessel wall.

Conclusion: S100A13 released from VSMCs and other CLEC-2 ligand on the surface of VSMCs may contribute to thrombus formation at the site of plaque erosion and stent thrombosis in vivo.

Disclosure of Interest: None declared.

PO420-MON

Platelet-Fibrin Thrombi can kill *Stenotrophomonas maltophilia*

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Background: Platelet α -granules contain antimicrobial polypeptides, collectively designated platelet microbicidal proteins (PMP). PMP processing and release take place upon platelet activation by agonists. Gram-negative, multi-drug resistant *S. maltophilia* is an opportunistic pathogen, particularly among hospitalized patients. *S. maltophilia* infections are associated with high morbidity and mortality in immuno-compromised individuals.

Aims: Can platelets kill *S. maltophilia*, especially upon thrombin-induced thrombus formation?

Methods: Prior to activation with thrombin (0.1 U mL⁻¹) for 30 min at 37 °C, incubation of washed platelets, PRP (9 μ L, 2.5×10^5 platelets μ L⁻¹) or PPP (control) was performed with 1 μ L of *S. maltophilia* culture (2×10^8 colony forming unit mL⁻¹) for 4 min at RT. To determine killing of bacteria within the thrombi, clots and aggregates were lysed with 0.05 U mL⁻¹ protease K and 1% saponin for 10 min at RT. Before plating onto soya extract solid medium, probes were sonicated for 30 s. Controls were performed without addition of thrombin, protease K or saponin. Platelet-bacteria interaction, complete lysis of platelet-bacteria aggregates and killing of bacteria were visualized by confocal laser scanning microscopy (LSM). For this purpose, platelets and bacteria had been fluorescently stained with cell tracker CMTMR, mepacrine, Syto 9, or propidium iodide, respectively.

Results: As shown by LSM, washed platelets can kill *S. maltophilia* in thrombin-induced platelet aggregates. To determine the amount of killed bacteria, aggregates were lysed by protease K and saponin. Neither protease K nor 1% saponin influenced the cfu. Thrombin-induced platelet aggregates reduced cfu of *S. maltophilia* by $> 80\%$ ($P < 0.05$), while unstimulated platelets reduced cfu by 40%, both in comparison with otherwise equally treated PPP.

Conclusion: Platelets can kill *S. maltophilia*. Platelet-fibrin thrombi may form a trap for bacteria and thus promote killing of bacteria by platelets.

Disclosure of Interest: None declared.

PO421-MON

Engineering platelets for the delivery of RNA

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Background: Local delivery of therapeutics to sites of damage within blood vessels remains a major challenge in managing hemorrhage and cardiovascular disease. Nature has in part overcome this challenge by using platelets as delivery vehicles for small molecules and biological macromolecules that regulate coagulation and inflammation. Platelets receive a large number of messenger RNAs (mRNAs) and microRNAs (miRNAs) during formation from megakaryocytes, and have been

shown to alter protein expression in nearby endothelial cells through the release and transfer of RNA-containing microparticles. The natural role of platelets as delivery vehicles provides strong motivation for utilizing these cells as carriers of RNA-based therapeutic agents.

Aims: My project aims to use nanoliposomes to directly introduce RNA to platelets for the release and delivery of this RNA to other cells types following platelet activation, leading to altered gene expression in the recipient cells.

Methods: To complete this aim, purified, *in vitro* transcribed RNA was encapsulated within nanoliposomes, and incubated with platelets *ex vivo* to allow for uptake. Flow cytometry and quantitative PCR were used to measure the platelets' ability to endocytose and release the liposomes and RNA.

Results: Using this approach, I have shown that exogenous RNA can be delivered to platelets via endocytosis, and this RNA is controllably released during platelet activation. Both premature miRNA and mRNA can be delivered to and released by the platelets. Future work will focus on testing whether this RNA can be transferred to and utilized in the target cells.

Conclusion: This is a novel approach for the delivery of RNA to platelets, and has many potential long-term applications depending on the type of RNA encapsulated within liposomes. For example, it may be possible to use this technology to create platelets that can be used to treat and heal atherosclerotic plaques by delivering miRNA against mediators of inflammation.

Disclosure of Interest: None declared.

PO422-MON

Inhibition of sorbitol dehydrogenase increases platelets adhesion in mice treated with fidarestat

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Background: A previous proteomic study on platelets obtained from 3 spontaneous and recurrent proximal deep vein thrombosis (DVT) patients, 1 sibling and 1 neighbor for each patient showed that sorbitol dehydrogenase (SORD) was present only in platelets from patients and absent in siblings and neighbors.

Aims: We analyzed the gene expression of SORD in the same group of individuals, as well as the influence of the inhibition of SORD with fidarestat in mice platelets adhesion.

Methods: qPCR was performed on platelets RNA after exclusion of leukocyte contamination. Fidarestat (10 mg kg⁻¹) or 5% arabic gum was added to 24 C57Bl6 mice. Platelets were washed in Krebs buffer and adjusted to $1.2 \times 10^8 \mu\text{L}^{-1}$ before addition to plates pre-covered with 50 $\mu\text{g mL}^{-1}$ fibrinogen, with and without 50 mU of thrombin. Adherent platelets were calculated comparing results to a standard curve.

Results: On qPCR, the gene expression of SORD was increased in patients (1x) in comparison to siblings (0.51x), but similar to neighbors (0.77x). The adhesion of platelets unstimulated was similar between both groups of animals (fidarestat:41.6; 2.5–91.6; SD=25.9; controls:45; 9.8–91.3; SD:22.6; *P*:0.69), but after stimulation with thrombin, platelets from treated animals presented increased adhesion (median:74.4; 42.9–98.8; SD:24) when compared to controls (median: 43.9; 13.6–48.2; SD:12.1; *P*:0.01).

Conclusion: The increase of SORD gene expression in patients in comparison to all the controls can corroborate the involvement of this protein on DVT physiology. The inhibition of SORD with fidarestat increased platelets adhesion only after stimulation with thrombin. Our hypothesis is that SORD may be a protective protein based on the increased gene expression, protein levels and adherent properties after

thrombogenic stimuli, and may be an attempt to reduce platelet function and minimize the damage.

Disclosure of Interest: None declared.

PO423-MON

The RAP1-GAP RASA3 prevents clearance of circulating platelets

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Background: The small GTPase RAP1 is critical for platelet activation and thrombus formation. RAP1 activity is controlled by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). Proteomics and transcriptomics studies identified RASA3 as the most abundant RAP1-GAP in platelets.

Aims: To elucidate the contribution of RASA3 to megakaryocytes and platelet function.

Methods: Mice lacking RASA3 in all cells (*Rasa3*^{-/-}) or in megakaryocytes/platelets only (*PF4-Cre*⁺ *Rasa3*^{fl/fl}) were generated. Mice with very low RASA3 expression due to a missense mutation (p.H794L, *Rasa3*^{hlb/hlb}) were also identified. The effect of impaired RASA3 expression on platelet production and survival was evaluated.

Results: *Rasa3*^{-/-} and *PF4-Cre*⁺ *Rasa3*^{fl/fl} mice exhibited severe thrombocytopenia, embryonic hemorrhage, and high embryonic/perinatal lethality. *Rasa3*^{hlb/hlb} mice exhibited less severe thrombocytopenia and normal survival. Megakaryocyte numbers were elevated in the bone marrow and spleen of *Rasa3*^{hlb/hlb} mice. Proliferation and *ex vivo* proplatelet formation were comparable between wild type and *Rasa3*^{hlb/hlb} megakaryocytes. However, circulating platelets were pre-activated and their half-life was strongly reduced. Concomitant genetic deletion of CalDAG-GEFI restored platelet count and platelet half-life in *Rasa3*^{hlb/hlb} mice. Cleared *Rasa3*^{hlb/hlb} platelets predominantly accumulated in the spleen and liver, but both splenectomy and macrophage depletion with clodronate-liposomes had only a mild effect on the peripheral platelet count of *Rasa3*^{hlb/hlb} mice.

Conclusion: These studies identify RASA3 as a critical inhibitor of platelet activation. Impaired expression of RASA3 causes premature platelet activation and clearance in mice. Preliminary studies suggest that platelet clearance occurs independently of macrophages in the spleen and liver. Other potential clearance mechanisms are currently under investigation.

Disclosure of Interest: None declared.

PO424-MON

Biomechanics of blood clot contraction

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Background: Despite the importance of blood clot contraction, it is a poorly understood mechanical process driven by forces generated by

platelet contractile proteins. The process consists of the volume shrinkage of the initial size of the platelet-fibrin meshwork resulting in compression and deformation of red blood cells (RBCs).

Aims: Elucidation of the mechanical response and mechanism of contracting clots with and without RBCs through the use of a mathematical model.

Methods: The kinetics of clot contraction was quantified using an optical analyzer system, and the mechanical response and contractile force was measured using rheometry. The overall mechanical response of the contraction process was modeled as a three-element active poroviscoelastic material.

Results: In contraction of unclamped clots, an increased volume fraction of RBCs critically lessened the rate and degree of contraction (by ~30% at 50% vs. 0% volume fraction of RBCs). In clamped contraction, the addition of 50% volume fraction of RBCs resulted in a ~60% increase in the contractile forces generated by the platelet-fibrin meshwork when compared to platelets and fibrin alone. Modeling showed that due to the length scale of poroelastic diffusivity, clot contraction was not a result of poroelastic behavior, but rather can be considered an active viscoelastic material. Our model revealed that this phenomenon occurred due to the presence of an element, consisting of RBCs and fibrin, in series with the active contractile element. This caused a shift in the stall stress of the system and an increase in the overall contractile force generated by the activated platelets.

Conclusion: Collectively, these results show that contracting blood clots behave as an active viscoelastic material and support a novel concept that RBCs are not simply a bystander in the formation and properties of clots and thrombi, but may be important in hemostasis since they influence the maximum active stress generated by platelets.

Disclosure of Interest: None declared.

PO425-MON

Reversal of the antithrombotic properties of quercetin-3-Rutinoside with the B' domain of PDI in a mouse thrombosis model

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Background: We demonstrated that inhibition of PDI activity by quercetin-3-rutinoside blocked both platelet accumulation and fibrin generation in a mouse thrombosis model. However, the binding site of quercetin-3-rutinoside on PDI is not known. PDI has four domains: a, b, b', a' of which a and a' contain the active site.

Aims: Here, we identify the binding site of quercetin-3-rutinoside on PDI and determine whether this region can serve as an antidote to reverse the antithrombotic effect of quercetin-3-rutinoside in a live mouse.

Methods: We generated 12 recombinant PDI fragments: 4 one-domain fragments (a, b, b', a'), 3 two-domain fragments (ab, bb', b'xa'), 3 three-domain fragments (abb', abb'x, bb'xa'), 2 four-domain fragments (abb'xa', abb'xa'c). We performed binding studies to characterize the interaction of quercetin-3-rutinoside with the PDI fragments. The fragment containing the quercetin-3-rutinoside binding site was studied in an *in vivo* laser-induced thrombosis mouse model to evaluate reversal of the antithrombotic effect of quercetin-3-rutinoside.

Results: Using a fluorescence enhancement-based assay and isothermal calorimetry, we show that quercetin-3-rutinoside directly binds to the b'x and bb' fragments of PDI with a 1:1 stoichiometry and a K_d of 11.5 and 10.4 μM respectively but not to fragments lacking the b' domain. Reductase activity of fragments containing the a or a' domains were only inhibited by quercetin-3-rutinoside if they contained the b' domain. Infusion of the b'x fragment of PDI completely reversed quercetin-3-rutinoside-mediated inhibition of thrombus for-

mation in a mouse model. This b'x fragment alone does not possess any reductase activity and does not affect thrombus formation *in vivo*.

Conclusion: Quercetin-3-rutinoside binds directly to the b' domain of PDI. The b' domain of PDI has the potential for use as an antidote to reverse the antithrombotic effect of quercetin-3-rutinoside in the event of bleeding complications.

Disclosure of Interest: None declared.

PO426-MON

Platelet in rats with acute renal failure present hyperreactivity in vitro

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Background: Rhabdomyolysis-induced myoglobinuric acute renal failure accounts for about 10–40% of all cases of acute renal failure (ARF). The function of the coagulation system itself is already profoundly changed in patients with acute renal failure, as they are prone to episodes of bleeding. On the other hand, they may also develop excessive formation of thrombi. Platelet, a vital role in the coagulation system, may be a major contributor.

Aims: This study was designed to investigate the platelet dysfunction in glycerol-induced ARF in rats.

Methods: SD rats were randomly divided into two groups. The rats in group I were injected with 50% glycerol (10 mL kg⁻¹, intramuscularly), and the rats in group II was given equal saline served as control. After 3 days of glycerol injections, the blood samples and kidney tissues were harvested for platelet function test and pathology analyses. Platelet aggregations in response to different concentrations of agonists were measured in washed platelet (collagen, CRP:collagen related peptide, thrombin, U46619) and platelet-rich plasma (ADP) with optical aggregometry. At the same time, ATP release from platelet granules in two groups stimulated by collagen was measured.

Results: Glycerol administration resulted in an increase in the mean creatinine (Cr), urea nitrogen (BUN) and urea acid (UA) in plasma and caused severe histologic damage. Platelet max aggregation induced by different agonists in ARF rats were significantly increased compared to the control. The ATP release in model group was also much higher than control group.

Conclusion: In this study we found that rats with ARF showed elevated platelet reactivity in response to collagen, CRP, thrombin, U46619, and ADP compared to the control at low dose. These results is the first time to present the aberrant platelet function in ARF rat, and can also contribute to our understanding of the clinical complex of ARF patient.

Disclosure of Interest: None declared.

PO427-MON

Factor XI-dependent platelet thrombin generation induced by thrombin is regulated by CD36

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Background: CD36, a member of the class B scavenger receptor family, is abundantly expressed on the platelet surface. Binding of platelets to thrombospondin-1 (TSP-1) via CD36 has been shown to enhance collagen-dependent thrombus formation and stabilization *in vitro* and

in vivo. Oxidized low density protein binding to CD36 induces a hyperreactive platelet phenotype associated with pro-thrombotic and pro-atherogenic effects in mice. However, the role of CD36 in thrombin generation on thrombin-stimulated platelets is not clear.

Aims: Investigating the role of CD36 in thrombin generation and activation of human platelets stimulated with thrombin in vitro.

Methods: Thrombin generation was assessed in recalcified platelet-rich plasma from healthy or CD36-deficient volunteers by calibrated automated thrombography using alpha-thrombin as trigger. Activation of platelet GPIIb/IIIa, granule secretion, binding of TSP-1, von Willebrand factor (vWF), coagulation factors and phosphatidylserine exposure were analyzed by flow cytometry. Blocking antibodies, annexin-V and tirofiban were used for functional studies.

Results: Platelets from a proband with CD36-deficiency or blocked by anti-CD36 antibody FA6-152 showed significantly decreased thrombin peak and endogenous thrombin potential as well as GPIIb/IIIa activation, granule secretion, binding of TSP-1, vWF and factor VIII, IX, X in response to low or moderate thrombin concentration compared to control platelets. In contrast, annexin-V did not affect platelet-dependent thrombin generation and procoagulant activity was not affected of CD36-deficient or FA6-152-treated platelets. While treatment of platelet-rich plasma with tirofiban or abciximab resulted in significantly reduced thrombin-triggered thrombin generation, additional inhibition was observed when FA6-152 treatment was combined.

Conclusion: These results indicate a new role of CD36 in the amplification of feedback activated factor XI-driven thrombin generation on thrombin-stimulated platelets.

Disclosure of Interest: None declared.

PO428-MON

Deletion of PI3K P110A results in enhanced primer-mediated regulation of platelet function and thrombosis

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Background: Platelet hyperactivity is a contributing factor in the pathogenesis of cardiovascular disease, and can be induced by elevated levels of circulating peptide hormones, such as the insulin-like growth factors (IGF-1 and IGF-2), insulin and thrombopoietin (TPO). We and others showed that primer-mediated platelet function is PI3 kinase dependent and initial pharmacological studies have suggested a role for p110 α .

Aims: To explore the role of p110 α in enhancement of platelet function by insulin, IGF-2 and TPO using a platelet-specific p110 α knockout murine model in conjunction with pharmacological inhibitors.

Methods: We examined integrin activation (JON/A binding), P-selectin exposure, thrombus formation (in vitro flow studies), rap1b activation (RalGDS pull-downs), calcium signalling, Akt phosphorylation and PIP3 formation (mass-spec approach) in platelets from wild-type and p110 α KO mice.

Results: We demonstrate that agonist-stimulated signalling to Akt/PKB as well as integrin α IIB β 3 activation, α -granule secretion, aggregation and thrombus-formation over collagen were unaffected by p110 α deletion. As expected, the primers insulin, IGF2 and TPO induced significant enhancements in integrin α IIB β 3 activation, α -granule secretion and *ex vivo* thrombus formation in wild-type platelets. Surprisingly, deletion of p110 α did not reduce, but significantly enhanced, the effect of the primers on platelet function and thrombosis. The enhanced priming in the p110 α KO was not mediated by elevations in calcium, rap1b activation, changes in PIP3 levels or Akt phosphorylation and could not be mimicked by the p110 α inhibitor PIK75 in wild-type platelets, suggesting that the phenotype of the p110 α KO is not due to compensation or absence of kinase activity.

Conclusion: p110 α deletion results in enhanced platelet priming in a manner that is independent of lipid kinase activity, suggesting that p110 α itself may limit the extent of platelet priming.

Disclosure of Interest: None declared.

PO429-MON

Profiling oxylipins released by GPVI-activated platelets and the effect of inhibitors of Cyclooxygenase-1 and 12-Lipoxygenase

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Background: Platelet activation results in liberation of arachidonic acid (AA) and metabolism by cyclooxygenase-1 (COX-1) and 12-lipoxygenase (12-LOX) to oxylipins. Whilst the role of COX-1 in platelet function is well established the role of 12-LOX is unclear.

Aims: To identify the oxylipins released from GPVI-activated platelets and investigate the effect of COX-1 and 12-LOX inhibition on this profile.

Methods: Washed platelets from healthy donors, resuspended in HEPES-buffered saline (HBS) pH7.4 with 2 mM Ca²⁺ and 20 μ g mL⁻¹ Hirudin, were incubated at 37 °C for 10 min with 50 μ M or 150 μ M baicalin or esculetin to inhibit 12-LOX, or 500 μ M aspirin to inhibit COX-1, before activation with 0.5 μ g mL⁻¹ CRP-XL. Platelet releasate was isolated by centrifugation, oxylipins were extracted using a hexane-based method and resuspended in 70% ethanol for analysis by LC-MS/MS.

Results: Washed platelets from healthy donors, resuspended in HEPES-buffered saline (HBS) pH7.4 with 2 mM Ca²⁺ and 20 μ g mL⁻¹ Hirudin, were incubated at 37 °C for 10 min with 50 μ M or 150 μ M baicalin or esculetin to inhibit 12-LOX, or 500 μ M aspirin to inhibit COX-1, before activation with 0.5 μ g mL⁻¹ CRP-XL. Platelet releasate was isolated by centrifugation, oxylipins were extracted using a hexane-based method and resuspended in 70% ethanol for analysis by LC-MS/MS.

Conclusion: This is the first study to fully profile oxylipins released from activated platelets and compare the effect of COX-1 and 12-LOX inhibition. In addition to aspirin's known role in inhibiting TXB2 formation, this study has, for the first time, shown the contribution of COX-1 to 15-HETE, 9- and 13-HODE formation. These results also highlight the importance of 12-LOX in the production of all the measured HETE's and HODE's. Further study into the action of these 12-LOX metabolites in atherothrombosis is warranted.

Disclosure of Interest: None declared.

PO430-MON

Role of hydrodynamic shear mediated platelet deformation on cell tethering, translocation and activation

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Background: During thrombus formation, platelet-VWF binding follows a multistep process that involves "transient cell capture", "platelet translocation", "cell activation" and finally "stable platelet adhesion". The VWF-GPIIb α bond is a critical regulator of this process as it controls capture and translocation under hydrodynamic shear.

Aims: We tested the hypothesis that in addition to bond mechanics, platelet deformation is also an important regulator of the cell adhesion cascade.

Methods: Platelet binding studies were conducted in parallel plate flow chambers using three systems: i. normal deformable human platelets; ii. non-deformable GPIb α bearing beads (GPIb α -beads); and iii. deformation-tunable erythrocytes that have surface bound GPIb α (GPIb α -RBCs). To alter the nature of cell/particle interaction, fluid media viscosity and substrate composition were varied.

Results: Platelet translocation on immobilized VWF followed a bell-shaped profile with peak cell accumulation and lowest translocation velocity at 4 dyn cm⁻². Experimental data collected upon varying media viscosity were aligned when accumulation and rolling velocity were plotted as a function of wall shear stress, rather than shear rate. As opposed to platelets, GPIb α -beads exhibited a monotonic decrease in bead accumulation and increase in rolling velocity. The rolling phenotype of GPIb α -RBCs was similar to that of platelets. Increasing the media osmolarity in this case from 0.3 to 0.6 Osm decreased the peak translocation shear stress from 1 to 0.5 dyn cm⁻², presumably due to increased cellular deformation. The shear stress for peak platelet rolling was also decreased from 4 to 1 dyn cm⁻² upon truncating the VWF D'D3-domain, a structural motif that shields GpIb α access to the A1-domain.

Conclusion: The data are consistent with a model where flow-induced platelet morphology changes reduce platelet rolling velocity on VWF by increasing the number of GPIb α -VWF bonds under shear.

Disclosure of Interest: None declared.

PO431-MON

The activating effects of shear stress gradients on thrombus formation

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Background: The complex shear stress conditions induced by intraluminal thrombi are known to be pro-thrombotic but remain poorly characterised. Recent studies suggest that local gradients of shear stress (SSG), created by the 3D geometry of platelet aggregates, are more pro-thrombotic than constant shear stress. Von Willebrand Factor (VWF) interaction with GPIb α is critical for thrombus formation under high shear stress and this interaction is modulated by the governing shear stress conditions.

Aims: To investigate the pro-thrombotic effects of SSGs on VWF-platelet binding and subsequent thrombus formation.

Methods: Platelet aggregation and VWF-GPIb α binding was measured using custom-made microfluidic flow channels by perfusing citrated whole blood over a collagen surface. SSGs were created through stenotic channel segments or via microspot thrombosis. We tested monoclonal antibodies against VWF A1 domain 6G1, 5D2, CR1 and CR3 for their capacity to inhibit VWF-dependent platelet aggregation under constant shear and SSGs.

Results: SSGs were greatest at the upstream edge of individual platelet aggregates and coincided with the highest amount of VWF binding. Platelet aggregation at the upstream edge of a micro spot thrombus, where SSG are maximal, was increased 1.6-fold relative to aggregates at the downstream edge. VWF dependent platelet aggregation was enhanced under shear stress gradients compared to constant shear stress. Compared to control IgG, 5D2 and CR1 abolished platelet aggregation under SSG and constant shear conditions up to 120 dyn cm⁻². However, SSGs from 30 to 240 dyn cm⁻² (mean 120 dyn cm⁻²) could overcome the inhibitory effect of blocking the VWF domains occupied by 6G1 and CR3.

Conclusion: SSGs are pro-thrombotic by increasing the binding of VWF to platelet aggregates. Elucidating the pro-thrombotic effects of shear stress gradients on the activation state of VWF may lead to the identification of new drug targets that are shear sensitive and selective for pathological thrombus formation.

Disclosure of Interest: None declared.

PO432-MON

Membrane estrogen receptor (MER) – protease activated receptor (PAR) cross talk modulates platelet function

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Background: 17 β -estradiol (E2) plays vital roles in reproductive function, energy homeostasis and stress responses as well as emerging roles in hemostasis and thrombosis through classical estrogen receptor (ERa and ERb) pathways as well as novel estrogen receptors such as a putative G α -coupled membrane estrogen receptor (Gq-mER). Using a yeast 3-hybrid screen of a pancreatic library, we discovered that mERs may partner with protease activated receptors (PARs) and found that Gq-mER interacts functionally with PARs in human platelets, suggesting a specific route for E2 signaling to influence platelet function.

Aims: We tested the hypothesis that the PARs synergize with a putative Gq-mER to activate multiple downstream signaling cascades that regulate platelet activation.

Methods: To elucidate the cross-talk between the Gq-mER and PAR signaling pathways in platelets, biochemical, cell biological and platelet functional assays were carried out using platelets stimulated with E2 as well as the Gq-mER selective agonist STX.

Results: To elucidate the cross-talk between the Gq-mER and PAR signaling pathways in platelets, biochemical, cell biological and platelet functional assays were carried out using platelets stimulated with E2 as well as the Gq-mER selective agonist STX.

Conclusion: Our results show that platelets bind to the Gq-mER selective agonist STX and suggest that platelet Gq-mER activation synergizes with PARs to contribute to functional modulation mediated by estrogen signaling. Future work will define the exact signaling pathways and platelet functional responses regulated by Gq-mERs and establish insights into the hormonal modulation of hemostasis.

Disclosure of Interest: None declared.

Platelet disorders I

PO433-MON

Platelet CD34 expression is increased in families with distinct GFI1B mutations: utility as a screening test

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Background: Inherited macrothrombocytopenias (IMT) are a clinically heterogeneous group of disorders, often causing moderate to severe bleeding tendencies. Diagnostic steps have recently been outlined by the ISTH-SSC and include phenotypic as well as genetic based methods. However, despite these, often complex investigations, many conditions remain undiagnosed.

Aims: To investigate the ability of CD34 platelet expression (measured by flow cytometry) combined with next generation sequencing (NGS) to delineate IMT caused by mutant transcription factors (TF) such as GFI1B since Monteferrario et al recently reported increased platelet CD34 in patients with the GFI1B p.Gln287* mutation.

Methods: Informed consent was obtained from patients and their blood collected for platelet testing and DNA sequencing. NGS was performed using MiSeq Illumina instrument and a 19 gene panel with 96% coverage.

Results: Totally 95 individuals from 61 families were included. A mutation in *GFI1B* (c.G503T:p.C168F) was detected in 3 patients from 2 families. This mutation located in Zn finger 1 may not be critical for DNA binding but may destabilize the Zinc-finger loop. Additional family members were tested and the presence of the G503T mutation demonstrated by Sanger sequencing confirmed the autosomal dominant transmission. All carriers presented with moderate macrothrombocytopenia and their platelets expressed CD34 in contrast to wild-type platelets. Furthermore, when compared to the T247 fs-*GFI1B* mutant we previously described, CD34 expression was only moderately increased. CD34 was not detected on platelets of patients with other TF mutations such as GATA1, FLI1 and RUNX1.

Conclusion: In two families, we have identified a c.G503T-*GFI1B* mutation which causes autosomal dominant macrothrombocytopenia. To date, only platelets with *GFI1B* mutations appear to express CD34, a feature not seen with other TF mutations. Importantly, the trend seen in CD34 expression may be indicative of phenotypic severity.

Disclosure of Interest: None declared.

PO434-MON

A case of MYH9 disorders caused by a novel mutation (P.K74E)

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Background: *MYH9* disorders are rare hereditary autosomal dominant disorders characterized by macrothrombocytopenia and Döhle body-like cytoplasmic inclusion bodies. *MYH9* disorders also encompass Alport manifestations such as nephritis, sensory deafness and cataract. These clinical phenotypes vary according to the location of mutation in *MYH9*.

Aims: Determination of the mutation which causes *MYH9* disorders and characterization of clinical phenotypes in a *MYH9* disorders patient.

Methods: Description of a case:: History: The patient is a 40 years old woman. She was diagnosed as thrombocytopenia in her childhood. When she was 35 years old, she developed renal failure, and after 3 years, hemodialysis was instituted because of progression of her renal failure.

Family history: Her mother was diagnosed as thrombocytopenia, renal failure and deafness. Her uncle and grandfather were diagnosed as renal failure. Her cousin was diagnosed as thrombocytopenia.

Results: Laboratory data: WBC $9.3 \times 10^9 \text{ L}^{-1}$, RBC $4.65 \times 10^{12} \text{ L}^{-1}$, Hb 136 g L^{-1} , Plt $55 \times 10^9 \text{ L}^{-1}$, BUN 370 mg L^{-1} , Cre 86.2 mg L^{-1} . Prothrombin time, activated thromboplastin time, and platelet aggregation tests were all within normal limits.

Peripheral blood smear: by May-Giemsa staining we confirmed giant platelets and Döhle body-like cytoplasmic inclusion bodies in her neutrophils. Immunofluorescence staining for non-muscle myosin heavy chain IIA (NMMHC IIA) revealed abnormal NMMHC IIA localization in her neutrophils.

Genetic analysis: p.K74E in exon 1 was identified. This mutation is located at the head domain of NMMHC IIA.

Conclusion: NMMHC IIA sequence alignments nearby K74 are highly conserved residues among species. K74 is located near the SH1-helix in the head domain of NMMHC IIA. It was previously reported that mutations in SH1-helix such as p.W33C, p.S96L, p.R702C result in *MYH9* disorder with Alport manifestations. Therefore, we diagnosed her as *MYH9* disorders caused by the mutation p.K74E of the *MYH9* gene.

Disclosure of Interest: None declared.

PO435-MON

Calr mutations are associated with lower platelet and endothelial activation than the JAK2 mutation in essential thrombocythemia

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Background: Patients with *JAK2*-positive essential thrombocythemia (ET) have an increased frequency of thrombosis and a prothrombotic phenotype characterized by marked blood cell and coagulation activation.

Aims: Recently, a lower tendency to thrombosis has been reported in ET patients harboring mutations in the calreticulin (*CALR*) gene, but the mechanisms underlying this finding are unknown.

Methods: On that basis, platelet turnover and membrane or soluble markers of platelet, leukocyte, endothelial, and coagulation activation were compared in 44 patients with *JAK2*-positive ($n = 23$), *CALR*-positive ($n = 12$) or non-mutated ET ($n = 9$) and matched healthy individuals.

Results: At diagnosis, patients with *CALR* mutations were significantly younger and showed lower hemoglobin levels than those with the *JAK2* mutation-positive patients. Compared with controls, ET patients had increased platelet turnover or platelet, leukocyte and coagulation activation and those *CALR*-positive patients had lower MESF expression of P-selectin following collagen activation (12.6 ± 2.7 vs. 14.7 ± 3.4 , $P = 0.044$). The three groups of patients showed similar leukocyte and coagulation activation. However, *CALR*-positive patients, as compared with *JAK2*-positive patients, displayed significantly lower baseline (6.9 ± 1.2 vs. 8.0 ± 1.5 , $P = 0.009$), thrombin- (10.2 ± 2.2 vs. 11.9 ± 2.8 , $P = 0.045$) or epinephrine-induced (7.9 ± 2.0 vs. 9.4 ± 3.0 , $P = 0.049$) platelet P-selectin MESF expression and lower levels of soluble CD40 ligand (0.5 ± 0.2 vs. 0.9 ± 0.4 ag/platelet, $P = 0.001$) and von Willebrand factor antigen (88.9 ± 18.4 vs. 123.3 ± 33.1 IU dL⁻¹, $P = 0.003$).

Conclusion: This different prothrombotic phenotype, with less platelet and endothelial activation in *CALR*-positive ET, might contribute to the lower frequency of thrombotic complications in these patients as compared with those *JAK2*-positive ET.

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Disclosure of Interest: None declared.

PO436-MON

Different microtubule abnormalities in two cases of macrothrombocytopenia associated with tubb1 variants

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Background: Autosomal dominant macrothrombocytopenia (MTP) can result from genetic variants in the *TUBB1* gene encoding β 1-tubulin, the major constituent of microtubules (MT). The consequences of different mutations for MT structure are not well characterized

Aims: We have examined platelets from 2 patients with a missense F260S and a novel R282* stop gain codon in *TUBB1*. MT organization and the presence of different subtypes (α - β - and acetyl-tubulin) was analyzed and correlated with the platelet morphology of the two *TUBB1* variants.

Methods: The missense and stop gain codon mutations of *TUBB1* were identified by the BRIDGE-Blood Platelet Disease program using whole exome sequencing. EM, IF, and Western blotting were performed to analyze the different forms of tubulin in resting platelets or under depolymerizing conditions. Platelet spreading on a fibrinogen (Fg)-coated surface was also studied.

Results: In both cases the thrombocytopenia was moderate. Depending on the *TUBB1* mutation we found different abnormalities of the platelet MT. As described by Kunishima et al (2014) in platelets with a F260S mutation, a reduced α - and β -tubulin content was detected by IF and Western blot which was reminiscent of a decreased MT number. In contrast, for the R282* mutant, α - and β -tubulin were increased as was the acetylated form; F-actin was also increased. In both cases, the platelets were large and round, with distended channels characteristic of the F260S missense mutation giving a spread platelet appearance on fibrinogen. For the R282* variant with more rigid MT, platelet morphology is in favor of constraints in membrane movement.

Conclusion: In conclusion, different mutations affecting *TUBB1* result in moderate MTP and differently modify the platelet cytoskeleton with both a decreased or increased presence of β -tubulin and MT with altered number and stability, respectively. These results showing opposing abnormalities for MT content and stability suggest that platelet size does not directly depend on the MT content.

Disclosure of Interest: None declared.

PO437-MON

Soluble glycoprotein VIf A potential biomarker for disease activity and platelet reactivity in acute gout

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Background: Patients with gout or high serum urate (sUA) are at high risk of cardiovascular (CV) mortality. We previously demonstrated increased platelet reactivity in blood from patients with inflammatory arthritis (IA). Platelets amplify inflammation in the joint in IA via the collagen receptor, glycoprotein (GP)VI followed by the production of proinflammatory platelet microparticles. When platelets are activated,

the GPVI receptor is shed and is detectable in the plasma as soluble GPVI (sGPVI).

Aims: Our hypothesis was that sGPVI would be raised in patients with active gout compared to stable gout versus healthy controls. We also compared sGPVI levels in those with acute gout versus acute calcium pyrophosphate (CPP) arthritis.

Methods: Following ethics approval and informed consent, serum samples were taken from patients with active gout ($n = 12$). These samples were compared with patients with CPPD ($n = 5$), chronic gout ($n = 17$) and healthy controls ($n = 19$). Demographic data were measured. ESR, CRP, fibrinogen and sUA were assayed. Plasma GPVI samples were centrifuged at 720 g and then 20000 g to ensure that no platelets or platelet derived microparticles were present in the sample and sGPVI levels were measured by ELISA.

Results: Mean serum GPVI was similar between CPPD and chronic gout ($7.028 \pm 2.8 \text{ ng mL}^{-1}$ vs. $6.258 \pm 2.5 \text{ ng mL}^{-1}$ respectively), but significantly higher in acute gout patients ($9.808 \pm 4.5 \text{ ng mL}^{-1}$; $P < 0.05$). Serum GPVI was significantly higher in acute gout versus healthy controls. ($3.807 \pm 1.3 \text{ ng mL}^{-1}$). Mann-Whitney U test showed serum GPVI levels were significantly higher in those with acute gout versus chronic gout ($P < 0.03$).

Conclusion: Our data shows that serum GPVI levels are significantly higher in those with acute gout versus those with chronic gout, CPPD and healthy controls. This highlights the role of serum GPVI as a marker of both disease activity and platelet reactivity in acute gout. Platelet hyper-reactivity in patients with acute gout likely contributes to increased CV events in these patients.

Disclosure of Interest: None declared.

PO438-MON

Personalized reference intervals for platelet count reduce the prevalence of unexplained thrombocytopenia and increase that of reactive thrombocytosis in elderly people

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Background: The reference interval for platelet count currently in use in most Western countries is $150\text{--}450$ or $150\text{--}400 \times 10^9 \text{ platelets L}^{-1}$. However, many studies indicated that women have more platelets than men and platelet count progressively decreases with ageing. Moreover, ethnicity-related differences have been identified.

Aims: To evaluate the outcome of a personalized reference range recently identified in a study involving 40 978 Italian healthy subjects and which takes into account age and sex: $165\text{--}473 \times 10^9 \text{ platelets L}^{-1}$, regardless of gender, under 15 years of age; $136\text{--}436$ and $120\text{--}369 \times 10^9 \text{ L}^{-1}$ in women and men, respectively, between 15 and 64 years; and $119\text{--}396$ and $112\text{--}361 \times 10^9 \text{ L}^{-1}$ in women and men, respectively, over 64 years (PLoS One 2013;81:e54289).

Methods: The new personalized reference range was applied retrospectively, in parallel to that presently in use of $150\text{--}450 \times 10^9 \text{ L}^{-1}$, to a series of 925 consecutive Italian patients admitted to a department of Internal Medicine. The investigated population was mainly composed of elderly subjects (mean age 74 ± 14.2 years).

Results: The prevalence of thrombocytopenia was 21.3% with the traditional reference range and 16.9% with the personalized one. Switching from thrombocytopenic to non-thrombocytopenic affected in most cases subjects without any apparent cause of reduced platelet count, whose number dropped from 67 to $37 \times 10^9 \text{ L}^{-1}$. Also a few patients with chronic liver disorders turned from thrombocytopenic to non-thrombocytopenic.

The prevalence of thrombocytosis increased from 9.1% with the traditional range to 12.2% with the personalized range, and switching always occurred in patients with inflammatory disorders.

Conclusion: The most relevant effect of personalized reference intervals for platelet count was the reduction by nearly half in the proportion of subjects with an unexplained form of thrombocytopenia. Using the new range is expected to prevent many subjects to receive unnecessary test, this benefiting both involved people and the health system.

Disclosure of Interest: None declared.

PO439-MON

MYH9 disease: identification of two mutations associated with uncommon clinical phenotype

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Background: MYH9 is a rare autosomal dominant syndrome caused by mutations in the gene encoding for the heavy chain of non-muscle myosin (NMMHCIIA), characterized by macrothrombocytopenia from birth. Some patients present neurosensorial hearing loss, cataracts and/or nephropathy. Large series have suggested a genotype-phenotype relationship in MYH9: mutations at residue R702 show the most severe phenotype for deafness and early development of nephropathy, while changes at R1165 or E1841 have low risk of kidney disease (Mol Genet Genomic Med 2014; 2: 297–312; Hum Mut 2014; 35: 236–47).

Aims: To characterize MYH9 disease in two macrothrombocytopenic patients with variable clinical presentation.

Methods: Clinical status, blood cell count, leukocyte inclusions, and MYH9 sequence were explored in both patients.

Results: Patient 1 showed macrothrombocytopenia ($20 \times 10^9 \text{ L}^{-1}$) and Döhle bodies in leukocytes, but no extrahematological defects. Patient 2 had thrombocytopenia ($35 \times 10^9 \text{ L}^{-1}$), leukocyte inclusions, bilateral neurosensorial hearing loss, and a rapidly progressing renal failure requiring renal transplant before the age of 40 year. His two daughters also show macrothrombocytopenia and renal disease. Analysis of MYH9 identified mutations c.3493C> T [R1165C] and c.5521G> A [E1841L], respectively. Although in patient 1 we expected changes at residues E1841 or D1424, associated with low risk of extra-hematological defects, he carries a R1165C change, which has been suggested to increase the risk of hearing loss at a young age. Even more strikingly, in patient 2 with the clinical picture of severe Epstein syndrome, we found the E1841L change, precisely one of the two mutations that do not associate to severe organic defects.

Conclusion: These two cases reflect that not only genetic changes, but also other unknown factors might influence MYH9 phenotype, and support close monitoring even in MYH9 patients with mutations not frequently associated to extra-hematologic abnormalities. PI14/01956 and RECAVA RD12/0042/0050 (ISCIII-FEDER)

Disclosure of Interest: None declared.

PO440-MON

ACTN1-related thrombocytopenias (ACTN1-RT): phenotype/genotype of a series of 13 new cases

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Background: ACTN1-RT is a new group of rare inherited macrothrombocytopenias (MTs) caused by mutations in the ACTN1 gene encoding the alpha-actinin isoform 1 (ACTN1), a cytoskeleton protein. Till now, only isolated cases or small series have been reported, characterized by non-syndromic phenotype, low bleeding tendency and moderate decrease of platelet count.

Aims: To identify patients with ACTN1 mutation among a cohort of patients with MT of unknown origin and to analyse their phenotype and genotype.

Methods: Phenotype analysis combined clinical data, platelet count and quantitation of large platelets (size over half the size of red cells) on MGG smears. ACTN1 genotyping was performed by Sanger sequencing.

Results: Among a group of 130 MTs, 63 were already diagnosed with MYH9 mutation and 1 with TUBB1 mutation. The 66 MTs of unknown origin were included in the study. Sequencing identified 13 (10%) cases with 10 distinct ACTN1 mutations affecting 7 exons and 3 ACTN1 functional domains. Four mutations, p.R46W, p.R46Q, p.V105I, p.R738W were already known but 6 were novel, not enlisted in dbSNP databases, predicted in silico to have deleterious effect and located in conserved amino-acid residues. All the mutations were missense but one, a stop codon p.E718*. Recurrent mutations reported in this study and in the literature involve a CpG site, the most common mutational hotspot in human genome. In the 13 carriers, bleeding tendency was absent or mild. Thrombocytopenia was mild in all cases (platelet count (mean [range]: 85[39–124] G/L) but 1 (39 G/L). The percentage (mean, [range]) of large platelets was: 47.5 [17–83] including giant platelets (size over the size of red cells): (5.7[1–26]).

Conclusion: Our results confirm the mild bleeding phenotype in carriers of ACTN1 mutations, and increase the knowledge on ACTN1-RT. The prevalence of ACTN1 mutations strengthens the interest to include the systematic analysis of ACTN1 gene in the workup of MT.

Disclosure of Interest: None declared.

PO441-MON

Rock1 OR FLI1 mutations in patients with mild thrombocytopenia

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Background: Improvement in high-throughput sequencing methods allows now to increase knowledge on inherited thrombocytopenia etiologies.

Aims: Analysis of candidate genes in two trio families with a history of mild thrombocytopenia.

Methods: Whole exome sequencing (French national center of genotyping, Evry, France) was performed in the index cases and their two parents recruited in the bleeding and thrombosis exploration center, CHU Timone (Marseille, France) after eliminating all known etiologies. Candidate sequence variations were confirmed by conventional Sanger sequencing in the trio families and when possible in other relatives.

Results: Heterozygous *ROCK1* (Q1217E) was the only candidate variation in one family with macrothrombocytopenia (index case, platelet count: 108G/l, MPV: 15 fl). *ROCK1* is activated by filamin A; it activates the LIMK-cofilin pathway and may influence platelet production through its inhibiting effect on actin polymerization. The observed variation was in a highly conserved Pleckstrin Homology domain (PH). In the second trio family, a novel heterozygous mutation in *FLI1* encoding Friend leukemia integration 1 was identified in the proband and the father. *FLI1* is a member of the ETS family of transcription factors, which regulates genes expressed both during early and late megakaryocytopoiesis. The mutation (R144Q) is localized in the pointed domain which is a putative protein-protein interaction region. The symptoms were restricted to mild thrombocytopenia (index case: 131G/l, MPV: 13 fl) without bleeding. The persistence of Non muscle myosin heavy chain IIB (MYH10) in platelets was recently proposed as a biomarker for *FLI1* deletions. MYH10 was detected in the two carriers' platelets by western-blot but was absent in the mother and the other relatives.

Conclusion: *ROCK1* or *FLI1* alterations are defects that may be recognized in patients with mild thrombocytopenia.

Disclosure of Interest: None declared.

PO442-MON

Congenital thrombocytopenias and immune thrombocytopenic purpura: a misleading diagnosis

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Background: Congenital thrombocytopenias are a heterogeneous group of rare disorders and are often difficult to recognize, requiring a careful medical and laboratorial assessment. The clinical spectrum of congenital thrombocytopenias ranges from severe bleeding diatheses, recognized within the first few weeks of life, to mild conditions that may bleed only after significant haemostatic stimuli (surgeries) or remain undetected until adulthood. A late diagnosis and the absence of family history (in recessive forms or new mutations) can lead to a wrong diagnosis of acquired thrombocytopenia, particularly immune thrombocytopenic purpura (ITP), since the diagnosis of ITP is a diagnosis of exclusion. Thus, in the presence of refractory or atypical ITP, a congenital thrombocytopenia should be investigated.

Aims: Describe cases of congenital thrombocytopenias that were misdiagnosed as ITP.

Methods: A clinical and laboratorial analysis of 4 patients with an initial diagnosis of ITP, all previously treated with corticosteroids. The laboratory investigations included a platelet (PLT) count, size and morphology; closure time by PLT function analyser assay; aggregation studies with ATP secretion by lumi-aggregrometry; expression of PLT glycoprotein receptors by flow-cytometry; and measurement of von Willebrand factor.

Results: Subsequently and after reassessment, these cases were reclassified as: congenital platelet disorders (May-Hegglin Anomaly, Bernard Soulier syndrome and deficiency of collagen receptor) and platelet-endothelium interaction disease (type 2B von Willebrand disease).

Conclusion: The differential diagnosis between congenital and acquired forms of thrombocytopenia is crucial, focusing on diagnostic criteria, clinical presentations, genetic etiology and current medical management, in order to avoid unnecessary and potentially harmful treatments (corticosteroids, immunoglobulins or splenectomy).

Disclosure of Interest: None declared.

PO443-MON

Five new heterozygous mutations in the GPIB and GPIX are associated with the Bolzano phenotype

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Background: Bernard-Soulier syndrome is an autosomal recessive macrothrombocytopenia due to mutations in genes encoding the GPIb/IX/V complex with low expression at the platelet surface. To date, fifty homozygous or double heterozygous mutations have been described. In 2001, 6 Italian families were reported to carry one monoallelic base variation c.515C>T (A156V - Bolzano mutation) in the *GPIBA*. Since then, 103 new cases were reported and monoallelic mutations in the *GPIBA* (n = 3) and *GPIBB* (n = 3) genes were described with the same phenotype.

Aims: To search for GPIb/IX/V monoallelic mutations in patients suffering for macrothrombocytopenia without giant platelets (platelet size > red blood cell size) and without known molecular diagnosis.

Methods: We sequenced *GPIBA*, *GPIBB* and *GPIX* genes. Megakaryocyte maturation was studied in one patient. Blood CD34⁺ cells were cultured in serum free medium with thrombopoietin and stem cell factor.

Results: Monoallelic mutations in the GPIb/IX/V complex were found in 10 patients out of 6 families (mean platelet count = 103 ± 17G/L; mean platelet volume (MPV) = 13 ± 1.6 fL). None of who suffered bleeding diathesis. A slightly reduced ristocetin aggregation and GPIb/IX/V complex expression was noticed in few patients. We identified five new heterozygous mutations in genes of the GPIb/IX/V complex: 2 in the gene (*C33Y*, *N150R*), 2 in the *GPIBB* gene (*G43R*, *G43W*) and one in the *GPIX* gene (*A163D*). One of the studied families carries the A156V Bolzano mutation. The two *GPIBA* mutants were tested with different prediction algorithms (PolyPhen-2 and SIFT software) and appear deleterious or probably damaging. Megakaryocyte maturation studied in the N150R patient shows a delay in polyploidization.

Conclusion: *GPIBA*, *GPIBB* and *GPIX* genes require to be sequenced in all patients with mild thrombocytopenia, moderate elevation of the MPV, without or few giant platelets. Particular attention has to be paid to slight decreases in GPIb/IX/V expression and ristocetin aggregation.

Disclosure of Interest: None declared.

PO444-MON

Increase the number of GPIIb-IIIa and P2Y12 receptors in activated platelets – possible evidence for proteins' synthesis de novoSirotkina O^{1,2,3}, Andoskin P^{1,2}, Emelyanov A^{1,2} and Vavilova T³¹Molecular Genetic Technology Department, First Pavlov State Medical University of St.Petersburg; ²Human Molecular Genetic Laboratory, B.P. Konstantinov Petersburg Nuclear Physics Institute, Gatchina, Leningrad district; ³Department of clinical laboratory diagnostics, Federal North-West Medical Research Centre, St. Petersburg, Russia**Background:** Although platelets lack a nucleus or genomic DNA, they are able to translate mRNA into proteins de novo. The key platelets' receptors may be involved in the regulation proteins' synthesis and may reflect proteins' synthesis in platelet de novo.**Aims:** To investigate the modification in the number of platelets' receptors in activated cells.**Methods:** We investigated the native platelets from 50 healthy donors (mean age 38 ± 2). The number of fibrinogen receptors GPIIb-IIIa and ADP-receptors P2Y12 in resting and activated (by ADP or collagen) platelets were studied by flow cytometry and Western blotting. The quantity of IL-1β, the protein which synthesis de novo in activated platelet was demonstrated earlier, was also analyzed for verification of the used method.**Results:** The numbers of GPIIb-IIIa and P2Y12 receptors on platelet membrane were 14.4 ± 0.9 and 11.1 ± 1.1, respectively. The correlation between numbers of GPIIb-IIIa and P2Y12 receptors on platelet's membrane was detected ($R = 0.45$, $P = 0.03$). The quantity of receptors was higher in donors with high level of platelet aggregation. The number of GPIIb-IIIa was changed in activated platelets from 18.1 ± 2.8 to 23.2 ± 3.1 and from 17.7 ± 3.3 to 20.9 ± 3.4 after 15 min and 2 h incubation with ADP. The P2Y12 level was higher in activated by ADP platelets compared to resting cells according to results of Western blotting. The GPIIb-IIIa receptor plays a key role in the regulation of proteins' synthesis in platelets de novo. The process of proteins' synthesis was inhibited by selective GPIIb-IIIa blocker (Monafram). The P2Y12 level was lower in sample treated with Monafram: 14.0 ± 3.0 and 5.8 ± 0.5, 18.0 ± 3.7 and 9.3 ± 6.6, 13.5 ± 2.1 and 5.7 ± 0.1 for P2Y12 from 15 min, 2 h and 24 h incubations with ADP and ADP+Monafram, respectively ($P = 0.046$). This result was confirmed by Western blotting. The same results were shown for IL-1β.**Conclusion:** According to above mentioned we may conclude that GPIIb-IIIa and P2Y12 proteins were synthesized in activated platelets.**Disclosure of Interest:** None declared.

Pediatric thrombosis and hemostasis

PO445-MON

Risk factors and co-morbidities in adolescent thromboembolism are different than those in younger children

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Background: In adolescent thromboembolism (TE), variety of risk factors (RFs) and co-morbidities (CMs) are reported, though overall prevalence of these have not been evaluated.**Aims:** We hypothesized that the spectrum of RFs/CMs in adolescent TE differs from that reported in children overall and sought to review our experience at Texas Children's Hospital (TCH).**Methods:** Medical records of adolescents (ages 12–21 years), diagnosed with arterial or venous TE (AT or VTE) at TCH from 01/01/2004 to 01/01/2014, were retrospectively reviewed as approved by the IRB. Adolescents with superficial vein thrombosis only were excluded. Patients' clinical details were evaluated.**Results:** Sixty five adolescents (32 F, 33 M) met study criteria. Median age at diagnosis was 16 years (range 12–20 years). Fifty-eight (89%) had VTE, 1 (1.5%) had AT, 5 (8%) had both VTE and AT, and 1 (1.5%) had cutaneous microthrombi. Associated RFs/CMs included obesity (48%), CVL (26%), infection (26%), surgery (26%), autoimmune disease (23%), immobility (20%), anatomical abnormality (18.5%), cancer (7.5%), estrogen therapy (6%), tobacco use (6%), trauma (3%) and other medical conditions (12%). Prothrombotic hereditary RFs were present in 13/50 (26%) tested. Eleven patients (17%) had ≤ 1 and 54 (83%) had ≥ 2 identified RFs/CMs. Therapy included anticoagulants (100%), antiplatelet agents (6%), thrombolytic agents (25%) and interventional therapy (29%). Four were lost to follow up and 2 had no follow-up imaging. Of 59 with follow-up imaging, 56% had complete response, 25.5% had partial response, 8.5% had no change and 10% had progression. Fourteen (24%) had recurrent TE. Majority (78.5%) with recurrent TE had ≥ 2 RFs/CMs.**Conclusion:** Adolescent TE is often multi-factorial with majority having ≥ 2 RFs/CMs at diagnosis and at recurrence, suggesting the need for detailed evaluation for RFs/CMs in this population, which may in turn enable optimal therapy and institution of RF modifying strategies to prevent recurrence.**Disclosure of Interest:** None declared.

PO446-MON

Do preterm infants with patent ductus arteriosus (PDA) who fail indomethacin treatment have lower platelet counts?: a systematic review and meta-analysisMitra S¹, Paes B², Chan AKC² and on behalf of THIN group (Thrombosis & Haemostasis in Newborns)¹Division of Neonatology, Department of Pediatrics;²Department of Pediatrics, McMaster University, Hamilton, Canada**Background:** Indomethacin has been the drug of choice for closure of PDA in preterm infants. Failure of a primary course of indomethacin to close the PDA is not uncommon. Several observational studies have linked low platelet counts to indomethacin failure. Recent animal studies have also highlighted the potential role of platelets in PDA closure.**Aims:** To conduct a systematic review and meta-analysis of the association of platelet counts and indomethacin failure in preterm infants with PDA**Methods:** The authors searched MEDLINE, Embase, CINAHL and PubMed, abstracts and conference proceedings and contacted the primary authors of relevant studies. Studies were included if they reported the use of indomethacin for PDA closure, compared a group which failed indomethacin treatment versus a group which didn't and reported primary data that could be used to measure the association between platelet counts and indomethacin failure. Two reviewers independently screened the search results and assessed methodological quality using the Newcastle-Ottawa Scale. Results were expressed as mean difference in platelet counts and were calculated using a fixed effects model.**Results:** We identified 660 potentially relevant studies. Nine studies reported platelet counts with indomethacin failure, but five did not meet a priori data abstraction criteria. Four studies involving 669 preterm neonates with 139 cases of indomethacin failure were included. Platelet counts were found to be significantly lower in infants with failed indomethacin treatment [Mean difference – $25.07 \times 10^9 \text{ L}^{-1}$; 95% CI: -39.30×10^9 , $-10.84 \times 10^9 \text{ L}^{-1}$; $I^2 = 0$].

Conclusion: Platelet counts appear to be significantly lower in infants with indomethacin failure. Though these preliminary findings align with the pathophysiological role of platelets in PDA closure, more prospective cohort studies that consistently report platelet counts in prostaglandin inhibitor failure are needed so that a larger meta-analysis can be conducted to establish or refute a significant association.

Disclosure of Interest: None declared.

PO447-MON

Catheter directed thrombolysis as safe and effective therapy for proximal lower limb deep vein thrombosis in adolescents

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Background: There is limited evidence to support catheter directed thrombolysis (CDT) for proximal deep vein thrombosis (DVT) in paediatric practice. Beneficial in adults, it has the potential to reduce life-long morbidity of post thrombotic syndrome (PTS) in children and adolescents.

Aims: To evaluate the efficacy and safety of CDT in the management of proximal DVT in adolescents.

Methods: A retrospective review of proximal DVT in adolescents managed with CDT over an 18-month period in a single institution.

Results: Five cases were identified, 3 male, aged 15–16 years. Risk factors were: minor trauma, 3; oral contraceptive pill, 2; reduced mobility, 2; travel, 1; obesity, 1. 4 had an abnormal thrombophilia screen: protein S deficiency, 2; FV Leiden (R506Q) mutation, 2 (1 homozygous). One has congenital IVC anomaly.

CDT was performed a median of 8 days (range: 3 to 16) after onset of symptoms. 2 had a temporary IVC filter placed prior to CDT. The popliteal vein on the affected side was catheterized and thrombus injected with recombinant tissue plasminogen activator (rtPA) and mechanical thrombectomy performed. Infusion of rtPA at 0.5 mg h⁻¹ was continued via the catheter with concurrent systemic infusion of unfractionated heparin at 100–200units h⁻¹. Repeat venogram was performed daily. The infusions were continued until adequate thrombus clearance was achieved. 1 had complete clot resolution and 4 had residual minimal, non-occlusive thrombus. 4 required venoplasty for identified stenosis. One required red cell transfusion for bleeding from the catheter insertion site. Use of full-length support stockings was recommended for 2 years and all cases continue long-term anticoagulation with warfarin. At a median follow up of 15 months (range: 3–25) there were no recurrent thromboembolic events. One case has mild PTS with limb swelling/aching on exertion and a 3 cm discrepancy in calf circumference.

Conclusion: This case series supports CDT as safe and effective management of proximal lower limb DVT in adolescents.

Disclosure of Interest: None declared.

PO448-MON

Age-dependency of coagulation parameters during childhood. results of a multicenter study

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Background: Understanding of developmental hemostasis is critical to ensure optimal prevention, diagnosis, and treatment of hemorrhagic

and thrombotic diseases in children. As coagulation test results are known to be dependent on the reagents/analyzers used, it is recommended for each laboratory to define the age-dependent reference ranges by using its own technical condition.

Aims: To address that issue, a study was carried out in 6 centers using the same reagents and ACL TOP 500/700 analyzers (IL, Bedford, USA).

Methods: There were 1331 samples obtained from pediatric patients (895 M and 436 F) aged between 2 weeks and 17 Y. They were divided in 6 age groups: <1-month (*n* = 37), 1–5 months (310), 6–12 months (*n* = 162), 1–5 years (*n* = 482), 6–10 Y (*n* = 117) and 11–17 Y (*n* = 223). Every sample was tested for global assays (PT and aPTT) while specific assays were performed in at least 20 samples in each age group.

Results: aPTT was negatively correlated with age (*r* = -0.15, *P* < 0.01), with longer clotting times in younger children. PT was correlated with age (*r* = +0.61, *P* < 0.0001), with shorter clotting times in younger children, and the same applied to fibrinogen (Clauss) level (*r* = +0.47, *P* < 0.0001). Factor V (FV), FVIII, FX and FXIII levels were found roughly unchanged during childhood. In contrast, FII, FIX, FXI, and FXII levels were significantly decreased in the youngest children (<1 Y) before reaching adult values. The same applied to antithrombin, PC and PS, and plasminogen. Von Willebrand factor (RCO and antigen) was found elevated at birth and remained elevated during the first months of life before it normalized at 6 month. Interestingly, D-dimer levels were found elevated during childhood, mainly during the first 6 months of life and remained slightly elevated before reaching adult values during puberty.

Conclusion: These data suggest that most coagulation test results are highly dependent on age, mainly during the first year of life, and that age-specific reference ranges must be used to ensure proper evaluation of coagulation in children.

Disclosure of Interest: None declared.

PO449-MON

Clinical features in children presenting from the community with pulmonary embolism

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Background: Pulmonary embolism (PE) in children is uncommon, whilst recognition and diagnosis is often difficult. Current clinical decision rules for PE are based on adult populations and have not been validated in the paediatric population. The development of paediatric-specific clinical decision rules may enable clinicians to confidently deal with the diagnostic uncertainty of PE.

Aims: To identify and evaluate clinical features which distinguish PE in children presenting from the community.

Methods: We present a case-control study of patients presenting to The Royal Children's Hospital Melbourne (RCH) between November 2007 and February 2015. RCH is the major tertiary paediatric referral hospital for the state of Victoria, Australia. Cases were defined as having radiologically proven PE, ventilation-perfusion (VQ) scan with a high probability diagnosis or a positive computed tomography pulmonary angiogram (CTPA), whilst controls were patients with a clinical suspicion of PE, but found not to have PE on diagnostic imaging and clinical follow-up. Charts, electronic medical and imaging records of both cases and controls were reviewed and analysed. In addition, data

was sought from state-based coronial services of children who died in the community of PE during the study period.

Results: The number of cases and controls, their demographic data, and comparisons of their clinical and investigation findings will be presented.

Conclusion: This study compared children presenting to the emergency department with radiologically proven PE to a group of children with suspected PE but ultimately negative imaging studies, and identifies the clinical indicators that are most useful in identifying which patients require definitive imaging from those who do not.

Disclosure of Interest: None declared.

PO450-MON

Effects of maternal hypertension on neonatal hemogram in South-south Nigeria

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Background: Hypertension in pregnancy complicates about 4–22% of all pregnancies, resulting in fetomaternal complications and constitutes one of the major causes of maternal and fetal/neonatal morbidity and mortality. The fetal/neonatal complications are largely secondary to intrauterine hypoxia and uteroplacental insufficiency leading to hematological and biochemical derangements, intrauterine growth restriction, prematurity and bronchial dysplasia. Neonatal polycythemia, neutropenia and thrombocytopenia are notable sequela of maternal hypertension; however, this has not been evaluated in our population.

Aims: To determine the hemogram of neonates of hypertensive mothers using those of normotensive mothers as controls.

Methods: This was a cross-sectional hospital-based case control study. Cord blood from neonates of both hypertensive (cases) and normotensive mothers (controls) were sampled for hemogram using a 3-part autoanalyser. WBC differential was done manually. Data were analyzed using SPSS version 16.

Results: A total of 200 neonates were recruited comprising of 100 neonates of hypertensive mothers and 100 neonates of normotensive mothers. The mean hematocrit was higher in neonates of hypertensive mothers $51.1 \pm 9.0\%$ vs. 45.5 ± 7.5 respectively, $P < 0.001$. Their neutrophil and platelet counts were $4.5 \pm 3.1 \times 10^9 \text{ L}^{-1}$ and $157 \pm 106 \times 10^9 \text{ L}^{-1}$ vs. $5.4 \pm 2.7 \times 10^9 \text{ L}^{-1}$ and $212 \pm 88 \times 10^9 \text{ L}^{-1}$ ($P = 0.033$ and <0.001 respectively). The prevalence of polycythemia, neutropenia and thrombocytopenia was 8%, 15% and 38% in neonates of hypertensive mothers and 0%, 2% and 8% in neonates of normotensive mothers ($P = 0.007$, 0.002 and <0.001 respectively).

Conclusion: From our study, neonatal polycythemia, neutropenia and thrombocytopenia were significantly higher in neonates of hypertensive mothers. It is therefore advisable that the hemogram of neonates of hypertensive mothers be closely monitored from birth to prevent complications associated with these disorders.

Disclosure of Interest: None declared.

PO451-MON

Treatment with romiplostim in children. Incidence and quality of the response

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Background: Agonists of thrombopoietin have changed the way we treat ITP. However, the incidence of responses and their quality are not well determined in children.

Aims: To define the incidence of response to romiplostim in children.

Methods: We conducted an observational retrospective analysis of the pediatric patients treated with romiplostim between 2009 and 2014. All of them were diagnosed with refractory ITP. Their ages were from 8 to 16 and none of them had been splenectomized. Compassive use of romiplostim and an informed consent were needed. Children received treatment once weekly at $1 \mu\text{g kg}^{-1}$ and dose was increased according to platelet response. We defined a complete response (CR) as platelets $>100 \times 10^9 \text{ L}^{-1}$ and no bleeding symptoms for more than 6 months. Maintained response (MR) was defined as platelets $>50 \times 10^9 \text{ L}^{-1}$ for more than 3 months without bleeding symptoms. Irregular Response (IR) was defined as the inability to maintain stable platelet counts.

Results: A total of 5 patients were treated with romiplostim as second line treatment. There were no CR. 3 of them (60%) reached MR, 2 of them (40%) reached an IR. Mean duration of the MR was 14.3 months (12–18). Mean time of treatment with romiplostim was 15.2 months (6–27). The maximum mean dose was $7 \mu\text{g kg}^{-1}$ to obtain response. Mean time to reach stable platelet count $>50 \times 10^9 \text{ L}^{-1}$ was 8 weeks.

Conclusion: There seems to be a lower percentage of complete responses in the pediatric population, due to low number of patients and probably because higher doses of romiplostim could be needed for children to obtain response. Duration of MR was 14.3 months.

Mean time to reach stable response was 8 weeks. A faster response could be obtained if higher doses were administered and used to escalate. Clinical trials are needed in this area.

Mean time of treatment was 15.2 months. No increase of secondary effects was observed due to treatment. Romiplostim could be used as “bridge therapy” to splenectomy in refractory ITP of recent diagnose.

Disclosure of Interest: None declared.

PO452-MON

Off-label use of recombinant factor VIIA for diffuse pulmonary hemorrhage in children

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Background: Diffuse alveolar hemorrhage (DAH) is a rare complication but associated with a very high mortality. The current treatment options of corticosteroids, transfusions, and immunosuppressants have been limited and largely unsuccessful, and they can be accompanied by multiple complications. Both intravenous and intrabronchial administration of activated recombinant factor VII (FVIIa) have been reported.

Aims: We report four cases with DAH who were rescued with intravenous rFVIIa and one patient rescued with combination of intravenous and inhalational use of rFVIIa.

Methods: Our study included 4 pediatric patients with acute onset of DAH who admitted to our hospital between 2010 and 2014. The median age was 5.6 years, and patients' conditions during DAH were hypovolemic shock with idiopathic thrombocytopenic purpura, septic shock with chronic granulomatous disease (CGD) after hemopoietic stem cell transplantation, and two patients with sarcoma and Burkitt's lymphoma under chemotherapy. These patients were treated with intravenous rFVIIa concurrent with tranexamic acid, fresh-frozen plasma, and maintenance of the platelet count $100\,000 \text{ mm}^{-3}$.

Results: DAH stopped with markable increasing arterial oxygen saturation was observed in 3 patients after intravenous rFVIIa. The fourth case suffering from sustained hypoxia without clinical improvement responded to combined intravenous and inhalational use of rFVIIa. Three patients exhibited rapid improvement and were successfully weaned from ventilators, except one died due to following multi-organ failure.

Conclusion: Our presentation illustrates that while administration of rFVIIa for DAH shows theoretical promise and combination of intravenous and inhalational use of rFVIIa may be a potential method for rescue of refractory DAH; however, further clinical studies are needed.

Disclosure of Interest: None declared.

PO453-MON

Investigating the role of polyphosphates in neonatal hemostasis

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Background: Neonatal platelets, despite poor aggregability in-vitro, support thrombin generation as well as platelets from adults. PolyP, a high molecular weight polyphosphate released by platelets could explain this discrepancy. PolyP can activate factor XII, therefore, posing a physiological activator of the intrinsic coagulation system. Comparable thrombin generation in neonatal and adult samples at very low tissue factor concentrations implicates an intrinsic fraction that could be triggered by PolyP. Additionally, PolyP could circumvent thrombin induced platelet activation in the initiation phase by activating factor XII, thus, rendering thrombin responsiveness of neonatal platelets less crucial for rapid clot formation.

Aims: We wanted to evaluate the impact of PolyP on neonatal and adult thrombin generation.

Methods: Thrombin generation was measured using Calibrated Automated Thrombography without tissue factor in platelet-poor plasma from venous adult blood ($N = 20$) or cord blood ($N = 20$) with/without addition of a PolyP standard ($100 \mu\text{g mL}^{-1}$). Aliquots of PolyP-spiked samples were preincubated with phosphatase from calf mucosa ($0.05 \text{ U } \mu\text{g}^{-1}$ PolyP) or corn trypsin inhibitor ($50 \mu\text{g mL}^{-1}$). Additionally, thrombin generation was measured in platelet-rich plasma samples triggered by collagen or thrombin receptor activating peptide.

Results: Lagtime and t_{peak} was significantly more reduced by PolyP in cord blood samples than in adult samples. The effect was completely abrogated by preincubation with phosphatase or addition of corn trypsin inhibitor. Impact of collagen and thrombin receptor activating peptide in platelet-rich plasma was comparable in both study groups, and prevented by addition of corn trypsin inhibitor.

Conclusion: Dynamic parameters were shorter in cord blood samples arguing for higher thrombin generation rate due to lower levels of inhibitors. Higher PolyP-reactivity might offset lower platelet reactivity in neonates resulting in comparable thrombin generation via the PolyP pathway.

Disclosure of Interest: None declared.

PO454-MON

Rationale and design of AESOP: APIXABAN for prevention of deep vein thrombosis in pediatric patients with acute lymphoblastic leukemia or lymphoma treated with L-Asparaginase

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Background: Approximately 1/3 of pediatric oncology patients receiving chemotherapy including L-asparaginase (ASP) develop deep vein thrombosis (DVT). Current standard of practice does not incorporate anticoagulation for DVT prevention, as to date, there have been no adequately powered trials determining efficacy and safety of primary prophylaxis. Apixaban is a novel anticoagulant which offers distinct advantages in the pediatric oncology population including oral administration, no antithrombin requirement and limited drug-drug interactions.

Aims: To determine the efficacy and safety of Apixaban in DVT prevention in pediatric patients during induction chemotherapy including ASP.

Methods: The AESOP trial is a Children's Oncology Group multicentre randomised controlled open label Phase III trial being conducted in over 80 centres in 3 countries. A total of 500 children will be randomised to receive either Apixaban or no anticoagulation during induction chemotherapy. Eligible subjects are ages 1 to < 18 years with newly diagnosed acute lymphoblastic leukemia or lymphoma and a central venous catheter in place. During induction, subjects will be followed for symptomatic DVT, which will be confirmed by objective imaging. At the end of induction therapy, all subjects will be screened for asymptomatic DVT by ultrasound and echocardiography. Primary efficacy (DVT) and safety endpoints (bleeding) will adhere to ISTH recommendations (Mitchell et al JTH 2011). Assessment of all study endpoint results will be by a blinded central adjudication committee and adverse events by an independent data monitoring committee.

Results: The AESOP trial design will be described in detail. Projected activation date is March 2015 and the study is anticipated to require 3.5 years to complete patient accrual.

Conclusion: AESOP is the first adequately powered Phase III randomised controlled anticoagulation trial in children. The study will determine the efficacy and safety of Apixaban in prevention of DVT in pediatric oncology patients.

Disclosure of Interest: V. Rodriguez: None declared, S. O'Brien Consultant for: Bristol Myers Squibb, L. Sung: None declared, L. Ramirez Employee of: Bristol Myers Squibb, D. Li Employee of: Bristol Myers Squibb, L. Mitchell Consultant for: Bristol Myers Squibb.

PO455-MON

Use of prothrombin complex concentrates (PCC) in paediatric extracorporeal life support patients

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Background: Prothrombin complex concentrates (PCC's) are human derived plasma products containing coagulation factors II, VII, IX, X, Protein C and S. Current accepted use includes treatment of bleeding

and perioperative reversal in acquired factor deficiencies as seen with vitamin K antagonist's (VKA). PCC use has properties that are attractive to extracorporeal life support, including ventricular assist devices, such as small volumes, rapid delivery, and pathogen inactivation. Potential disadvantages are thrombotic complications. There are no previous reports of PCC use in this population.

Aims: To describe prothrombin complex concentrate use in paediatric extracorporeal life support patients.

Methods: University of Alberta Ethics Board approved. A retrospective review over a 5 year period (2009–2013) was performed to determine the clinical indication, dose, bloodwork (INR), and adverse events.

Results: Five patients between 1 month and 15 years of age received PCCs. All patients had either a complex congenital heart condition or cardiomyopathy. Three received PCC's for VKA reversal pre-operatively and two for uncontrolled hemorrhage. Dosing was based on 3 groups of weight/INR combinations with 0.6 mL kg⁻¹ ($n = 3$), 1.0 mL kg⁻¹ ($n = 1$) and 1.4 mL kg⁻¹ ($n = 1$) as final doses given (range of 2.5 mL–40 mL volume in total). INR data was incomplete; two patients with complete data had INRs pre-PCC of 2.8 and post-PCC INRs of 1.6. All patients received additional blood products for bypass or uncontrolled hemorrhage. There were no adverse events related to PCC use.

Conclusion: This case series is the first to describe PCC use in paediatric ECLS patients. Prothrombin Complex Concentrates offer an attractive alternative to fresh frozen plasma for urgent reversals in this population due to small volume, fast product delivery, and pathogen inactivation. This case series did not find any adverse events attributable to PCC's. Further investigation is warranted.

Disclosure of Interest: T. Noga: None declared, S. Nahirniak: None declared, J. Conway: None declared, H. Buchholz: None declared, M. Bauman: None declared, P. Massicotte: None declared, A. Bruce Consultant for: Novartis, Speaker Bureau of: Novartis.

PO456-MON

Antithrombin plasma levels and fibtem determination in children with acute lymphoblastic leukemia undergoing asparaginase treatment

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Background: Children with acute lymphoblastic leukemia (ALL) are currently treated with L Asparaginase (Asp). Coagulopathies frequently occur and guidelines for identification of patients at risk are not yet established. Routine coagulation tests do not identify clinically significant abnormalities. Antithrombin (AT) levels are the only reliable parameter for thrombotic risk. The Clauss method does not correctly detect very low levels of fibrinogen. Whole Blood Rotation Thromboelastometry (ROTEM) using FIBTEM can identify hypofibrinogenemia and hemorrhagic risk in surgical patients. Maximum Clot Firmness (MCF, normal value 9–25 mm) is the maximum amplitude in millimeters reached in FIBTEM and is used to assess the specific role of fibrinogen in whole blood clot formation following inhibition of the platelets by Cytocalasin D.

Aims: To analyze the hemostatic profile using AT and ROTEM in children with ALL.

Methods: Forty two children (25 males, 17 females) were diagnosed with ALL and treated with Pegylated LAsp (PEG Asp, 2500UI mq⁻¹ per dose). ROTEM, AT, prothrombin time (PT), fibrinogen and a platelet count was performed at 5 fixed time-points before and after each PEG Asp administration.

Results: Totally 798 AT and 706 FIBTEM tests were performed. Only values of MCF >9 mm had a linear correlation with fibrinogen levels.

Plasma fibrinogen values <1 g l⁻¹ were only found in 15% of cases with low MCF. The PT was prolonged in only 5 cases (MCF 4–8 mm). Supplementation with AT or fibrinogen concentrates was suggested for patients with AT<50% or MCF<2 mm and decided on a clinical basis.

Conclusion: The correlation between fibrinogen levels measured by Clauss and fibrinogen "function" measured by MCF ROTEM was shown only with higher MCF levels. The determination of fibrinogen function by ROTEM[®] is a more specific test to the conventional clotting profile in the management of Asp-induced coagulopathy in pediatric ALL.

Disclosure of Interest: None declared.

PO457-MON

Warfarin therapy in children: can families safely and efficiently monitor INRs at home?

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Background: Limited data are available on the safety and effectiveness of point of care (POC) international normalized ratio (INR) testing in children and young adults, and whether families can be taught to dose-adjust under practitioner guidance.

Aims: To evaluate in children and young adults the safety and efficacy of POC testing and dose adjustments by the family.

Methods: Part I of the study consisted of utilizing the CoaguChek XS[®] at home with dose adjustments made by the practitioner. Part II consisted of self-dose adjustments based on home INR results. Three cohorts were chosen: (1) new to warfarin, (2) established on warfarin, and (3) low-adherence. An education program was completed prior to enrollment. The primary endpoint was percent of time in the therapeutic range (TTR) and incidence of thrombotic and bleeding events.

Results: Of 124 eligible patients, 30 were enrolled. Median age was 16.4 years (range 4–25). Average TTRs for patients in cohorts 1 and 2 were 60% (SD 27%) and 73% (SD 18%), respectively ($P = 0.24$). Cohort 2: INR control was no different before and after transition to a home INR monitor (TTR 69% [lab] vs. 73% [home], $P = 0.74$). Cohort 1: adherence was 100% during home testing. Cohort 2: adherence to INR tests was 98% during lab testing and 100% during in-home testing. Cohort 3 failed to enroll. Of the 30 total enrolled patients, 12 were eligible for part II of whom 8 consented. During the 3rd-month family dose adjustment period, the TTRs of the 8 patients were not significantly different (76% for part I vs. 70% for part II, $P = 0.36$). None of the 15 family dose titrations were deemed to be incorrect by the practitioners. There were no bleeding or thromboembolic events in the study.

Conclusion: POC testing and family dose adjustment can be performed with safety and effectiveness in adherent families. Adherence to INR testing at home was at least as good as in-laboratory testing. Data suggest low-adherence patients are difficult to recruit for studies.

Disclosure of Interest: None declared.

PO458-MON

Prophylactic enoxaparin in young infants – are the currently recommended doses too low?

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Background: Young infants often require increased dosing of enoxaparin to obtain therapeutic anti-Xa levels. However scant data are available on prophylactic enoxaparin dosing in this age group. Our anticoagulation program retrospectively reviewed our experience with prophylactic enoxaparin dosing in infants with a corrected gestational age of less than or equal to 2 months.

Aims: To review and determine appropriate prophylactic enoxaparin dosing in young infants.

Methods: Data were obtained from the Boston Children's IRB-approved anticoagulation program data base of infants with the indication of "prophylaxis", location in the "neonatal intensive care unit" and fiscal year greater than 2008. Age, weight, enoxaparin dose per kilogram and anti-Xa levels were compiled. Initial dosing was according to the ACCP Chest guidelines (0.5 - 0.75 mg kg⁻¹ SQ BID) with goal anti-Xa 0.1 - 0.4 units mL⁻¹.

Results: Seven patients met the study entry criteria. Median age was 26 days (range 8 - 48). Median weight was 3.76 kg. Average initial enoxaparin dose was 0.68 mg kg⁻¹. Five of the 7 patients had anti-Xa levels determined. Two of the 5 anti-Xa levels were undetectable (<0.1 units mL⁻¹). The 3 detectable anti-Xa levels were 0.10, 0.13 and 0.14 units mL⁻¹.

Conclusion: These data support a change in prophylactic enoxaparin dosing for young infants, given the insufficient anti-Xa levels with currently-recommended dosing strategies. We have therefore now adjusted prophylaxis dosing in our institution to 0.85 mg kg⁻¹ q12 h for post-menstrual age (PMA) ≥37 weeks and age <2 months and 1.0 mg kg⁻¹ q12 h for PMA <37 weeks and age <2 months. These doses are half of our newly-adopted dose for therapeutic enoxaparin in young infants based on recently published pharmacokinetics data.

Disclosure of Interest: None declared.

PO459-MON

Thromboembolic events in children

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Background: The incidence of thromboembolic events in children has increased dramatically. Early detection of risk factors in children would minimize morbidity and mortality.

Aims: This study was aimed to analyze the data of 60 thromboembolic events in children who were followed up in our hematology-oncology clinic between 2000–2015, retrospectively.

Methods: Information of 60 patients were retrieved from patient files and from the records contained in the electronic information processing environment created after 2005. Patient characteristics as well as their ages, general data on the diagnosis, risk factors, management, and duration of the treatment were recorded. Patients with leukemia were excluded.

Results: Thirty five of the patients were males and 25 were females. The age range was between four months-16 years. Presenting ages of the patients were changing from one day to 12 years. Of the children 8 (13.3%) of the cases were neonates, 19 (31.7%) were infants less than 1 year old, and 33 (55%) were children over than 1 year old. Thromboembolic events were mostly located in central nervous system 37 (61.7%), deep venous system of the limbs 9 (15.1%), portal vein 4

(6.7%), renal vein 2 (3.3%), intracardiac 2 (3.3%), inferior vena cava 2 (3.3%), peripheral artery 2 (3.3%) and pulmonary embolism 2 (3.3%). Inherited risk factors were present in 38 (63.3%) of the children. Ten of the patients carried two risk factors (16.9%). MTHFR was the most common inherited risk factor. Acquired risk factors were present in 31 (51.6%) of the children. Trauma was the most common underlying risk factor. Acquired and inherited risk factors were present simultaneously in 13 (22%) of the patients.

Conclusion: Based on these data diagnosis, screening and treatment issues will be discussed.

Disclosure of Interest: None declared.

PO460-MON

Bivalirudin use in children on ECMO following Heparin failure

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Background: Extracorporeal membranous oxygenation (ECMO) is challenging in children. Although, unfractionated heparin (UFH) is used for circuit thromboprophylaxis it is not always successful. Bivalirudin (Bi) may be a safe and effective alternative.

Aims: To compare hemorrhage and thrombotic outcomes on UFH vs Bi.

Methods: Cohort study of children who were unable to maintain a patient circuit on UFH were transitioned to Bi. Anti Factor Xa levels (0.35–0.7 µ mL⁻¹) and PTTs (60–100 s) were targeted for patients on UFH or Bi, respectively. Surrogate measures for thrombosis (Plasma free hemoglobin, PFHg, component changes (circuit, oxygenator, and pump) were collected.

Results: Five children, aged 1 month to 12 years who failed UFH were transitioned to Bi. Mean days on UFH or Bi were 44 days and 9 days, respectively. UFH doses ranged from 10 to 48 µg kg⁻¹ h⁻¹ with Bi doses ranging from 0.01 to 1.1 mg kg⁻¹ h⁻¹ titrated to achieve the monitoring targets. Mean transfused PRBCs on UFH or Bi were 26 and 25 mL⁻¹ kg⁻¹ per day, respectively. Mean transfused platelets on UFH or Bi were 24 and 8 mL⁻¹ kg⁻¹ per day, respectively. Mean PFHg on UFH or Bi was 103 and 99 mg dL⁻¹, respectively. There were 0.05 circuit changes per ECMO day on UFH and 0.02 circuit changes per ECMO day on Bi. Oxygenator changes were 0.02 per ECMO day for UFH with no changes in the Bi group. No Pump changes occurred in either group.

Conclusion: Bi appears to be a safe and effective alternative for pediatric ECMO patients who have failed UFH. Importantly, there were less thrombotic complications including decreased PFHg levels and number of circuit and oxygenator changes in patients transitioned to Bi with no increase in hemorrhagic complications (platelet consumption). The limitations in this study are the small number of patients therefore; larger studies are required to confirm these findings.

Disclosure of Interest: M. Bauman: None declared, P. Massicotte Consultant for: NIH/NHLBI, A. Bruce: None declared, D. Granoski: None declared, L. Lequier: None declared, L. Ryerson: None declared

PO461-MON

Thromboembolic events in pediatric patients with cyanotic congenital heart diseases in Srinagarind hospital

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*Department of Pediatrics, Faculty of Medicine, Khon Kaen University, Srinagarind Hospital, Khon Kaen, Thailand***Background:** Patients with cyanotic congenital heart diseases might risk of thromboembolic events from multiple factors such as polycythemia, surgical procedures for symptomatic treatment.**Aims:** To review patients with cyanotic congenital heart diseases who developed TE.**Methods:** Seven patients who developed TE were retrospectively reviewed.**Results:** Seven patients are 3 males, 4 females with age range from 2 months to 16 years transposition of great artery (1), pulmonic atresia (1), tricuspid atresia(1), and pulmonic stenosis(PS)(1). Presenting symptoms include focal seizure (3), jaundice(1), heart failure with face swelling(1), massive ascites(1), coldness and pulseless of leg(1). Three patients post Fontan had clot at anastomosis(2) and Intrahepatic inferior vena ca thrombosis(1). Two patients post bidirectional Glenn (BDG) shunt developed BDG shunt thrombosis (1) and right MCA infarction (1). One patient post *Blalock-Taussig*(BT) shunt had sino-venous thrombosis. One patient with PS developed arterial thrombosis of leg post cardiac catheterization. One patient had arterial stroke and sagittal sinus thrombosis related with polycythemia, infection and iron deficiency anemia. Patients post BT shunt, Fontan and BDG shunt received aspirin for thromboprophylaxis. Anticoagulation is given including enoxaparin in 7 cases with incomplete resolution of thrombosis.**Conclusion:** Risks of TE in patients with cyanotic heart disease might be related to polycythemia, iron deficiency anemia, and surgical treatment. Thromboprophylaxis should be an important role of prevention of TE post-surgical treatment.**Disclosure of Interest:** None declared.

PO462-MON

Phenomenon of hypercoagulability in children with deep vein thrombosisGracheva M¹, Zharkov P¹, Seregina E¹, Sepoyan H¹, Poletaev A¹ and Ataulakhanov F^{1,2,3,4,5,6}¹*Federal Scientific Clinical Center of Pediatric Hematology, Oncology and Immunology named after Dmitry Rogachev;*²*National Research Center for Hematology;* ³*Department of Physics, Lomonosov Moscow State University;* ⁴*The Faculty of Biological and Medical Physics, Moscow Institute of Physics and Technology;* ⁵*Center for Theoretical Problems of Physicochemical Pharmacology RAS;* ⁶*Research Department, HemaCore LLC, Moscow, Russia***Background:** Cancer, persistence of a central venous catheter (CVC) and chemotherapy treatment are proven as risk factors for deep vein thrombosis (DVT) in children. Hypercoagulability is a phenomenon characterized by tendency to accelerate the process of clot formation in vitro. However, the relationship of this phenomenon with clinical outcome - thrombosis - is poorly understood.**Aims:** To evaluate the presence of coagulation activation markers in children with newly diagnosed deep vein thrombosis (DVT) using conventional and global coagulation assays.**Methods:** Twenty children on specific anticancer therapy in age of 7 months to 16 years (median 4.5 years), 8 girls and 12 boys were enrolled in this prospective observational study. DVT was confirmed by Doppler ultrasonography. Conventional coagulation tests – APTT,

INR, fibrinogen, D-dimer and global hemostasis test Thrombodynamics were used to assess coagulation status before anticoagulation treatment. Hypercoagulability was defined when APTT and/or INR were below reference range or when fibrinogen and/or D-dimer and/or clot growth velocity (V) in Thrombodynamics were above reference range.

Results: Among 20 patients (pts) CVC associated DVT was revealed in 16 pts. INR was in normal range in 16 pts (80%) and slightly increased in 4 pts (20%). Fibrinogen concentration was reduced in 8 pts (40%), normal – in 11 pts (55%), increased – in 1 pt (5%). APTT was prolonged in 4 pts (20%), normal- in 14 pts (70%), shortened – in 2 pts (10%). D-dimer concentration was increased in 14 pts (70%). V in Thrombodynamics was reduced in 2 pts (5%), normal – in 9 pts (45%), increased – in 9 pts (45%). Either D-dimer or Thrombodynamics were increased in most part of pts: hypercoagulability was revealed in 17 pts (85%) at least by one of these tests and in 6 pts (30%) – by both.**Conclusion:** Thrombodynamics and indirect marker of blood clotting, D-dimer, especially their combination are supposed to be perspective methods to reveal hypercoagulability in this cohort of patients.**Disclosure of Interest:** M. Gracheva: None declared, P. Zharkov: None declared, E. Seregina: None declared, H. Sepoyan: None declared, A. Poletaev: None declared, F. Ataulakhanov Shareholder of: HemaCore LLC, Employee of: HemaCore LLC.

PO463-MON

Impact of inherited and acquired prothrombotic defects (PD) on the development of symptomatic thromboembolism (STE) in children with acute lymphoblastic leukemia (ALL) treated according to the Dana-Farber Cancer Institute (DFCI) 05-01 All-Therapy ProtocolAthale U¹, Laverdiere C², Nayiager T³, Delva Y-L⁴, Foster G⁵, Thabane L⁶ and Chan A¹¹*Pediatrics, McMaster University, Hamilton;* ²*Pediatrics, Ste. Justine Hospital, Montreal;* ³*Pediatric Hematology/Oncology, McMaster Children's Hospital, Hamilton;* ⁴*Pediatrics, Ste. Justin Hospital, Montreal;* ⁵*Clinical Epidemiology and Biostatistics, McMaster University, Hamilton;* ⁶*Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, Canada***Background:** Role of inherited or acquired PDs in development of TE in patients (pts) with ALL is uncertain.**Aims:** To define the effect of PD [low protein C, S, antithrombin (AT); high Factor VIII and von Willebrand factor (vWF); gene polymorphisms of methylene tetrahydrofolate reductase (MTHFR), prothrombin (PT), Factor V Leiden (FVL); anticardiolipin antibodies (ACLA)], and other factors [age, ALL risk-group, asparaginase (ASP) type, total white cell and blast count] on the development of objectively confirmed sTE in pts with ALL.**Methods:** After institutional ethics approval consenting pts (aged 1- ≤ 18 years) with de novo ALL were enrolled on the study. Samples for PD, collected prior to ALL therapy, were tested centrally. PD was defined using age-adjusted standardized data. ALL diagnosis, therapy and sTE data were collected. Regression analyses evaluated relationship between risk factors and sTE.**Results:** Of 131 pts [mean age (range) 6.4 (1–17) years.; 70 boys, 66 standard risk, 36 had PEG ASP] 20 (15%) had sTE. Prevalence of FVL was 2.4%, PT 3.2% and MTHFR (T/T or C/T) 61%. Acquired or inherited PDs had no impact on risk of sTE. Compared to pts without, pts with sTE were significantly older (*P* 0.048), had higher hemoglobin (*P* 0.041), higher total (*P* 0.048) and blast count (*P* 0.02) at ALL diagnosis. Older age (Hazard ratio (HR) 1.1, *P* 0.03), ALL risk type (HR 3.0, *P* 0.025) and vWF at diagnosis (HR 1.63, *P* 0.049) affected TE-free survival. Presence of blasts had shorter, but non-sig-

nificant TE-free survival (HR 5.7, P 0.09). Pts with circulating blasts had higher median vWF levels than those without (1.48 vs. 1.24, Wilcoxon P = 0.038); blast count and vWF were significantly correlated (P 0.003).

Conclusion: In our cohort, presence of PD at the time of ALL diagnosis did not affect risk of sTE during therapy. Alongside older age and ALL risk category, higher vWF and circulating blasts at diagnosis may increase the risk of sTE. We recommend evaluating the role of these novel risk factors in development of ALL-associated TE.

Disclosure of Interest: None declared.

PO464-MON

Examining the feasibility of recombinant factor viia to compensate for physiological or drug-induced impairment of platelet function: an in-vitro study

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Background: Bleeding in neonates is a rare but serious event. Neonatal platelets exhibit poor functionality in standard aggregation testing and could, therefore, be responsible for bleeding episodes. The neonatal physiological condition could be comparable to adult patients undergoing antiplatelet therapy e.g. with eptafibatide. In cases of refractory bleedings recombinant factor VIIa (rVIIa) is a therapeutic option. This drug is indicated in hemophiliacs with inhibitor and patients with Glanzmann's thrombasthenia, but it is commonly used off-label as a procoagulant agent in various bleeding situations.

Aims: Aim of our experiments was to evaluate the impact of rVIIa in cord blood or adult samples incubated with eptafibatide in-vitro using thrombelastogram measurements.

Methods: Blood samples were taken from healthy, adult volunteers and from placentas immediately after birth (n = 5 each). Citrated whole blood was analyzed with/without incubation with eptafibatide in a final concentration of 1 $\mu\text{g mL}^{-1}$ for 15 min and/or rVIIa in a concentration of 3.5 $\mu\text{g mL}^{-1}$ before measurement. Assays were performed with a TEM coagulation analyzer (ROTEM 05). 40 μL saline containing tissue factor (final concentration 0.35 pmol l^{-1}) were added to 300 μL of whole blood to start reaction. Specific thrombelastogram parameters (CT, CFT, MCF and Alpha) were recorded and compared.

Results: Addition of rVIIa results in significantly shorter clotting times in both, adult and cord blood samples. Further there is a significant shortening of clotting times by rVIIa in samples spiked with eptafibatide. A significant prolongation of clotting time and clot formation time with eptafibatide is only present in cord blood samples. No remarkable influence on MCF and alpha can be observed by these drugs.

Conclusion: Our in vitro experiments indicate that rVIIa has procoagulant effects in adult as well as in cord blood, and could potentially be beneficial in conditions of drug-induced platelet impairment.

Disclosure of Interest: None declared.

PO465-MON

Assessing the association of FVIII and abo blood group with deep vein thrombosis in pediatric oncology patients

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Background: FVIII and ABO blood group have been associated with deep venous thrombosis (DVT) in adults. However, to date, no studies have been done in pediatrics.

Aims: To investigate the relationship of FVIII and ABO blood group in association with DVT in pediatric oncology patients.

Methods: A case control study in survivors of childhood cancer recruited in 5 Canadian centres. Cases consisted of survivors who had experienced a DVT while being treated for pediatric cancer (n = 78). Controls were survivors who did not experience DVT (n = 178). Clinical information including age at diagnosis and chemotherapy were collected. Genotyping of blood group was done by single nucleotide polymorphisms (SNP) analysis identifying O and non-O blood groups using 3 SNP: rs8176746, rs505922, rs8176719. FVIII activity levels were measured on a STA compact. Logistic regression models were used to estimate the association between FVIII levels and ABO group with DVT. The multivariate regression included an interaction between FVIII and ABO group, adjusted for centres, age at cancer diagnosis and asparaginase. The odds ratio (OR) by 0.1 unit change in FVIII levels was reported.

Results: FVIII levels were significantly increased in cases $1.5 \pm 0.5 \mu\text{mL}^{-1}$ versus controls $1.2 \pm 0.4 \mu\text{mL}^{-1}$ (P < 0.001). There were significantly more Non-O blood group in the cases 68.8% when compared to controls 51% (P = 0.015). In univariate analysis, both FVIII (OR=1.15; 95% confidence interval [CI]: 1.07–1.23) and Non-O blood type (OR=2.2; 95% CI: 1.2–4.1) were associated with DVT. When multivariate analysis was performed with both FVIII and blood group, only FVIII remained significant (OR=1.2; 95% CI: 1.1–1.3; P = 0.002) and Non-O blood group was no longer significant (OR=1.7; 95% CI: 0.8–3.6; P = 0.18). In multivariate analysis assessing the effect of FVIII by blood type, FVIII level was associated with DVT only in patients with blood O type (OR=1.5; 95% CI: 1.2–1.8).

Conclusion: Increased FVIII levels are independently associated with DVT in pediatric oncology patients.

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Post-thrombotic syndrome

PO466-MON

Denser clots predispose to post thrombotic syndrome

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Background: Post thrombotic syndrome (PTS) is a chronic sequel of deep vein thrombosis (DVT). Denser fibrin clot structure and increased resistance to fibrinolysis is reported in DVT and other thrombotic disorders. The role of fibrin structure in PTS is unknown.

Aims: Assess the fibrinolytic potential and clot structure in PTS.

Methods: Patients with a history of DVT were included in a case-control study: patients with PTS (cases $n = 30$) and without PTS (controls $n = 30$), and 30 apparently healthy individuals (HI) without venous thromboembolism (VTE) or venous insufficiency were enrolled. Medical ethical approval of the committee of the Maastricht University Medical Centre, and written informed consent from all subjects was obtained. Fibrinolysis and clot structure were assessed by turbidimetric assays, permeation, and confocal microscopy. Fibrinogen was measured by Clauss and fibrinogen γ' by ELISA.

Results: We observed a significant trend of decreasing maximum turbidity from HI (median 0.52 [IQR 0.46–0.62]), to controls (0.49 [IQR 0.41–0.55]), to cases (0.46 [IQR 0.39–0.49]) $P = 0.020$. Fibrinogen was lower in patients (cases+controls) (3.69 g L⁻¹ [IQR 3.31–4.26]) compared to HI (4.17 [IQR 3.69–4.65]) $P = 0.041$. Patients with recurrent VTE had lower turbidity and permeation than patients with one episode of VTE ($P = 0.008$, $P = 0.047$), differences remained but lost significance after exclusion of patients on anticoagulant treatment (A/C). After adjustment for confounders, use of A/C showed an independent association with reduced turbidity, permeation, and increase in fibrinogen. There were no differences in lysis time, confocal microscopy, or fibrinogen γ' .

Conclusion: Lower maximum turbidity, indicating thinner fibers and denser clots, was found in patients with PTS. Our data further indicate that patients with recurrent VTE might have denser clots. Denser clots may predispose for PTS as they may result in poor recanalization, outflow resistance, and venous hypertension.

Disclosure of Interest: None declared.

PO467-MON

Practice variation in the treatment of DVT and prevention of post thrombotic syndrome between internists and vascular surgeons: a Canadian survey of physician practices

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Background: Recommendations for using anticoagulants and endovascular thrombus reduction (ETR) strategies for the treatment of deep

vein thrombosis (DVT) and preventing post thrombotic syndrome (PTS) vary between different specialties.

Aims: To determine whether there is a difference in the approach to management and prevention of DVT/PTS between internists and vascular surgeons.

Methods: A self-completed electronic survey with case scenarios was distributed to members of Thrombosis Canada and the Canadian Society for Vascular Surgery from Aug.-Oct. 2014.

Results: Of the 235 contacted, 84 (36%) answered the survey (51 internists and 33 surgeons). For management of ilio-femoral DVT, internists were less likely than surgeons to recommend ETR (45 vs. 86%; $P = 0.0005$), even for scenarios when a patient expressed concern about PTS. In patients with popliteal DVT, both internists and surgeons favour the use of anticoagulation as first line treatment, although surgeons were less likely to prescribe new oral anticoagulants (vs. warfarin) compared to internists (6 vs. 65%; $P < 0.0001$). A similar proportion of internists and surgeons would recommend the use of ETR in patients with popliteal DVT concerned about PTS. There was no difference in the prescription of graduated compression stockings (GCS) between groups ($P = 0.14$). Surgeons had better access to endovascular procedures for DVT than internists (97% vs. 53%; $P < 0.0001$). For surgeons, the primary barrier to prescribing ETR reported was MD unfamiliarity with indications for the procedure (27%), while for internists, it was lack of trained personnel (22%).

Conclusion: Our survey found significant practice variation between internists and vascular surgeons for the treatment of DVT and prevention of PTS; both groups continue to frequently use GCS despite the findings of recent studies. Future studies should address how to improve knowledge translation in order to ensure evidence based care is prescribed to patients with DVT regardless of the speciality of the treating physician.

Disclosure of Interest: None declared.

PO468-MON

Two weeks of low molecular weight heparin for isolated symptomatic distal vein thrombosis (twister study): interim analysis of first 100 patients – post thrombotic syndrome sub-study

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Background: Most deep vein thrombi (DVT) originate from a small calf vein (distal vein) thrombus, however 80–90% of distal DVT lyse spontaneously and are probably of minimal clinical consequence. The treatment and management of isolated distal DVT (IDDVT) thrombi is controversial, with treatments ranging from withholding of anticoagulation and a repeat compression vein ultrasound (CUS) of the leg veins in a week, to 3 months of full dose anticoagulation.

Aims: The TWISTER Study is a multicentre Australasian study examining the safety of 2 weeks of anticoagulation for a first episode of symptomatic IDDVT. The primary outcome is symptomatic recurrence of venous thrombosis DVT and pulmonary embolism, PE) within 3 months.

Methods: Patients with confirmed symptomatic distal DVT received 2 weeks of therapeutic anticoagulation (enoxaparin or rivaroxaban) and had a repeat CUS at the end of 2 weeks. If the patient was asymptomatic and there was no proximal DVT extension on CUS, treatment was stopped. If the patient was still symptomatic then treatment was continued for a further 4 weeks.

Results: An interim analysis of the first 100 patients was performed. Ninety-three received enoxaparin; 7 received rivaroxaban. The duration of anticoagulation was 2 weeks (± 1 week) for 77 patients, 6 weeks for 21 patients (with 2 receiving an additional 6 weeks at the investigator's discretion), and 3 months for 2 patients due to asymptomatic proximal extension on the 2 week CUS. There were also 5 extensions within the distal venous system, with all occurring whilst on full dose anticoagulation. There has been one VTE recurrence within 3 months (1.3%; 95%CI 0–3.8%), a distal DVT in the contralateral leg. All events were reviewed by three blinded adjudicators.

Conclusion: Our preliminary data suggest 2 weeks of therapeutic anticoagulation is sufficient for the majority of patients (77%) with a first episode of symptomatic IDVT, with a low rate of VTE recurrence within 3 months (1.3%).

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PO469-MON

Frequency of persistently abnormal echocardiography after acute pulmonary embolism

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Background: Successful treatment of acute pulmonary embolism (PE) should aim to reduce right ventricular (RV) dysfunction (RVD) and pulmonary hypertension (PH). Current literature does not allow an estimation of the frequency of persistent abnormalities, nor does it provide predictors of persistent echocardiography (echo) abnormality.

Aims: Determine from a large prospective, multi-national registry of patients with venous thromboembolism: 1) the frequency of persistent abnormality among patients who undergo repeat echo 2) What echo abnormalities predict persistence 3) Stratification of echo results by treatment.

Methods: Utilizing the Registro Informatizado de pacientes con Enfermedad TromboEmbólica (RIETE) registry, we queried all patients with PE and DVT (DVT was included because many patients with DVT who had an echo had high probability of PE) and RVD/PH was defined as RV hypokinesis, RV diastolic diameter >42 mm, or TAPSE <16 mm, or an estimated pulmonary artery systolic pressure >40 mm Hg.

Results: We examined 31,817 with PE and 26,917 with DVT and possible PE ($n = 53,738$). The echo was abnormal in 6058 (52.2%) at the time of diagnosis. Of the 352/738 (47.7%) patients with a persistently abnormal echo, the presence of abnormal TAPSE on initial echo was found to be a positive predictor of persistent echo abnormality (OR 1.8, 95% CI 1.42–2.51) while RV hypokinesis was not. With regards to patients with persistent TAPSE abnormality only 2 (0.5%) were treated with fibrinolysis, the remainder receiving LMWH or heparin.

Conclusion: In patients with confirmed or probable PE, an initial echo is frequently abnormal and the presence of an abnormal TAPSE may

predict an increased risk of persistent echo abnormality. Within 2 years, only 12% patients with an abnormal initial echo had a follow-up echo as standard care, and 47.7% had persistent RVD/PH. Few were treated with fibrinolysis. These data indicate the lack of knowledge of the natural history of RVD/PH from PE treated with anticoagulants alone.

Disclosure of Interest: None declared.

PO470-MON

Toll-like 9 gene expression in the post thrombotic syndrome, residual thrombosis and recurrent deep venous thrombosis: a case-control study

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Background: Post thrombotic syndrome (PTS) is a prevalent, but poorly understood, chronic complication of deep venous thrombosis (DVT). Animal models suggest that toll-like receptor 9 (TLR9) promotes thrombus resolution after acute DVT.

Aims: To assess whether the TLR9 expression is lower in patients with PTS and investigate whether this is associated with residual thrombosis (RT) and recurrence.

Methods: Patients with a history of DVT with PTS (cases), and without PTS after minimal 24 months follow-up (controls), were selected. Healthy individuals (HI) without DVT were included as reference. TLR9 mRNA expression in leukocytes was determined by qPCR and normalized to the housekeeping gene Succinate dehydrogenase complex subunit A using the $\Delta\Delta C_t$ method. The Kruskal-Wallis test was used to compare TLR9 expression levels, post-hoc Mann-Whitney U test was performed on significant Kruskal-Wallis results. Subanalyses were performed to explore the TLR9 expression in patients with and without RT and multiple DVT episodes.

Results: Eighty nine subjects were included: 30 cases, 30 controls and 29 HI. The median TLR9 expression was 0.45 (range 0.17–1.55), 0.39 (range 0.01–1.88) and 0.62 (range 0.14–1.27) in cases, controls and HI respectively ($P = 0.6$). RT was present in 25 (42%) patients with DVT. The median TLR9 expression was 0.39 (range 0.16–0.96) in these patients compared to 0.55 (range 0.01–1.88, $P = 0.1$) in those without RT. The median TLR9 expression was significantly lower in patients who had one DVT compared to patients with recurrent DVT, 0.37 (range 0.01–1.88) vs. 0.55 (range 0.23–1.55) respectively ($P < 0.01$).

Conclusion: No significant difference in TLR9 expression was found between cases, controls and HI. However TLR9 expression seems lower in individuals with DVT and RT, albeit not significant. Interestingly, in patients with recurrent DVT the TLR9 expression was significantly higher compared to patients with one DVT, suggesting ongoing activation of immunity in patients with recurrent DVT.

Disclosure of Interest: None declared.

PO471-MON

Patients' preferences regarding elastic compression stocking therapy for the prevention of post thrombotic syndrome

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Background: Elastic compression stocking (ECS) therapy is used for the prevention of post thrombotic syndrome (PTS) after deep vein thrombosis (DVT). Current evidence on its effectiveness is conflicting. Compliance, one of the most important determinants of effectiveness of ECS therapy, remained largely ignored in former studies.

Aims: To assess preferences concerning characteristics of ECS therapy in patients after DVT, in order to elicit determinants of compliance.

Methods: A discrete choice experiment (DCE) was conducted in patients 3 months after DVT enrolled in the IDEAL DVT study, a randomized controlled trial comparing 2 years ECS therapy to individually tailored duration of ECS therapy for the prevention of PTS. Nine unlabelled, forced, choice sets of two hypothetical types of ECS were presented to each patient. The ECS was described by 8 characteristics. A Bayesian efficient design was used to generate the choice sets. The data were analysed with multinomial logit models.

Results: The respondent sample consisted of 81% (200/247) of invited patients. Both treatment groups were equally represented. Significant determinants of preference were, in order of importance: 1. PTS risk reduction, 2. being able to put on the ECS independently, 3. duration of ECS therapy, 4. reduction of current complaints, 5. comfort of wearing, and 6. whether the ECS could be machine washed and dried. Costs and appearance of the ECS did not significantly influence preference. Patients were willing to increase therapy duration with one year for an additional PTS risk reduction of 10%. However, patients also accepted an increase in PTS risk of 29%, if they would be able to put on the ECS themselves.

Conclusion: For the patients in this study, risk reduction of PTS and the ability to put on the ECS without help are the most important characteristics. Patient education on PTS, but also enabling patient independence by improvement of and access to assistive aids for putting on/removing ECS, could maximize compliance in these patients.

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PO472-MON

Comparison of self-rated and professional-rated scores of the villalta scale for evaluation of the post-thrombotic syndrome in patients with proximal deep vein thrombosis

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Background: The Villalta scale (VS) is a widely accepted tool to assess development of post-thrombotic syndrome (PTS) in patients with deep

vein thrombosis (DVT), and includes scoring of both symptoms and signs. Traditionally it necessitates a clinical visit and scoring by a trained health-professional. Previous studies have shown good agreement between professional-rating of VS, but recently some researchers have administered the VS as a patient-rated questionnaire, even though this approach has not been validated.

Aims: Our aim was to evaluate the inter-rater agreement of the VS between patients and professionals in a clinical setting.

Methods: In a retrospective cohort of 61 patients with DVT treated with catheter-directed thrombolysis, professional-rated VS scores were compared with patient-rated VS scores 15–141 months after the diagnosis, accompanied with quality of life measures (QOL) (SF-36 and VEINES). We calculated Cohen's kappa (unweighted) as a measure of the inter-observer variability.

Results: The apparent prevalence of PTS (VS score ≥ 5) (69 vs. 52%, $P < 0.01$) and severe PTS (≥ 15) (20 vs. 5%, $P < 0.02$) was higher for patient-rated than for professional-rated scoring of PTS. The inter-rater agreement for PTS (PTS yes/no: Kappa 0.40, CI: 0.19–0.62) was lower than previously reported between professionals (Kappa 0.71). However, the correlation between total scores was strong (Spearman rho = 0.73, $P < 0.01$). The total score of signs was 1.8 ± 2.1 (mean $\pm$ SD) for professional-rated and 4.3 ± 4.0 for patient-rated ($P < 0.01$). Patient- and professional-rated VS were inversely related to both QOL measures ($P < 0.01$).

Conclusion: The prevalence of PTS was overestimated by patients, mainly due to higher ratings of PTS-related signs. Consequently, inter-rater agreement of the VS between professional and patients appeared to be lower than previously reported between professionals.

Disclosure of Interest: None declared.

PO473-MON

Is post thrombotic syndrome perceived to be a clinically relevant outcome?: A survey of physician priorities

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Background: Despite being a major source of morbidity in patients with deep vein thrombosis (DVT), the perceived importance of post thrombotic syndrome (PTS) as a clinical outcome by physicians is unknown.

Aims: To determine whether treating clinicians feel that PTS and severe PTS are a relevant endpoint.

Methods: An internet survey was distributed to the members of Thrombosis Canada and The Society of Vascular Surgeons of Canada. The survey was open from Aug. 2014-Oct. 2014. Responses were obtained on a 5 point Likert scale with references: 1(irrelevant), 3 (important) and 5(very important) or as multiple options 'yes', 'no' or 'uncertain'. Statistics calculated by Chi squared analysis.

Results: Of the 235 initially contacted 84 (36%) answered the survey (51 internists and 33 vascular surgeons). Any PTS was ranked as significantly less important as an outcome than recurrent DVT, PE during treatment, major bleeding, death, quality of life, venous ulceration and severe PTS (all $P < 0.05$). No major differences were noted between surgeons and non-surgeons in how they perceived the importance of outcomes except for recurrent DVT, which was ranked as less

important by surgeons as compared to internists ($P < 0.0001$). When presented with the results of the CaVenT study by Enden et al. (Lancet 2012), clinicians were divided on whether a 14% absolute reduction in PTS was clinically relevant, with similar numbers answering: Yes 38%, Uncertain 34%, and No 28% ($P = 0.59$). There was a trend towards surgeons being more likely to feel that a change of 14% was relevant compared to internists ($P = 0.14$).

Conclusion: Our survey determined that PTS in general is not appreciated as an important endpoint as compared to other clinical outcomes, however, severe PTS is considered important. Results of upcoming DVT treatment studies exploring more aggressive endovascular strategies to prevent PTS may not lead to a change in current practice unless they are adequately powered to show differences in more relevant clinical outcomes, including severe PTS.

Disclosure of Interest: None declared.

PO474-MON

Exercise limitation in chronic post-thrombotic iliofemoral vein obstruction

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Background: Chronic residual venous occlusion after deep vein thrombosis (DVT) may result in reduced exercise capacity

Aims: We aimed to study exercise physiology in post-DVT patients complaining of effort intolerance.

Methods: Exercise physiology was assessed by bimodality incremental symptom-limited cardiopulmonary exercise testing using leg or arm cycle ergometers. Subjects were 6 post-DVT patients with residual iliofemoral vein occlusion, complaining of effort intolerance, with no cause identified by standard workup; controls were 8 healthy volunteers. Leg vein obstruction was modeled by application of cuff tourniquets, inflated to 30 mmHg, to the thighs. Results are median (range).

Results: Leg exercise tolerance as measured by oxygen consumption at peak exercise (peak V'O₂) was reduced (46% predicted (36–67%) in patients vs 89% (77–130%) in controls, $P = 0.007$). Arm exercise tolerance was also reduced, but less severely than in the legs – the arm/leg ratio of peak V'O₂ was 1.14 (0.77–1.43) in patients vs 0.71 (0.6–1.0) in controls, $P < 0.003$. In controls, bilateral leg vein obstruction by application of tourniquets reduced peak V'O₂ in leg exercise to 76% predicted (55–106%) vs 89% (77–130%) in the standard test, $P < 0.05$.

Conclusion: The comparison of exercise capacity in arm vs leg exercise of post-DVT patients with residual iliofemoral vein occlusion, and the effect of experimental venous obstruction (thigh tourniquets) in healthy controls, suggest that reduced exercise capacity in the patients is caused by reduced venous return, as simulated in the controls. Chronic venous obstruction should be recognized as an exercise limiting factor.

Disclosure of Interest: None declared.

PO475-MON

Advancing the diagnosis of pediatric post-thrombotic syndrome: a foundation for the improvement and standardization of its measurement tools

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Background: The existing tools for diagnosis of post-thrombotic syndrome (PTS) in children have acknowledged limitations, stressing the need for their improvement.

Aims: To determine the diagnostic performance of different measurement techniques for signs and symptoms (S&S) of upper and lower limb (UL/LL) PTS in children.

Methods: Twenty-nine unique PTS S&S, collected from a survey of pediatric thrombosis experts ($n = 24$) and interviews of patients with PTS ($n = 16$), were defined and measured using state-of-the-art techniques in children at risk of PTS, ≥ 6 months after diagnosis of UL or LL deep vein thrombosis. S&S were grouped in 4 domains: 1) pain and other PTS symptoms, 2) limb edema, 3) skin signs, 4) PTS impact. The diagnostic performance of each S&S [area under receiver operating characteristic curve (AUC), positive/negative likelihood ratio (LR+/LR-)] for PTS diagnosis was determined for UL&LL (stratified analysis). Ethics approval and informed consent were obtained.

Results: UL stratum: 55 patients, median (IQR) age 6 (4) years, had a PTS frequency of no/mild/mod/severe = 56%, 29%, 13%, 2%. The best performing measurements per domain were: 1) PTS symptoms questionnaire (AUC 0.72, $P < .001$ /LR+12.9/LR-0.6); 2) truncated cone technique (AUC 0.72, $P = .002$ /LR+2.0/LR-0.7); 3) Boulden scale (AUC 0.72, $P = .001$ /LR+ 3.9/LR- 0.7); 4) endurance as per the self-perceived fitness questionnaire (SPFQ, AUC 0.61, $P = 0.03$ /LR+4.4/LR- 0.8)

LL stratum: 63 patients, median (IQR) age 7 (8) years, had a PTS frequency of no/mild/mod/severe = 46%, 40%, 14%, 0%. The best performing measurements per domain were: 1) adolescent pediatric pain tool (AUC 0.81, $P < .001$ /LR+ NE/LR-0.4); 2) bioimpedance ratio (AUC 0.66, $P = 0.02$ /LR+3.4/LR-0.9); 3) No discriminative ability for durometry, skin temperature and Boulden scale was found; 4) endurance as per the SPFQ (AUC 0.65 $P < 0.001$ /LR+9.4/LR- 0.7)

Conclusion: Measurement techniques for PTS S&S have different diagnostic properties; their evaluation will allow experts to standardize and improve PTS diagnosis in children.

Disclosure of Interest: None Declared.

PO476-MON

Fibrinogen characteristics help predict chronic thrombotic venous disease after acute deep vein thrombosis

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Background: Chronic thrombotic venous disease (CTVD) is defined by symptomatic venous obstruction that persists after anticoagulation for deep venous thrombosis (DVT).

Aims: We examined whether knowledge about the structure and function of fibrinogen, in addition to clinical data, improves the prediction of which DVT patients resolve venous patency vs. develop CTVD.

Methods: We compared cases with CTVD to controls who had complete resolution after DVT. Clinical data and fibrinogen structure, function and post-translational modification were examined as potential predictors of CTVD. A parsimonious Classification and Regression Tree (CART) model was constructed to differentiate cases from controls.

Results: A model that relied on a clinical history of prior venous thromboembolism and then characteristics of both the fibrinogen function (as measured by lysis time) and structure (as measured by post-translational sialylation of the fibrin B-beta chain) informally had the best performance characteristics relative to models with clinical data alone. The combined model correctly predicted CTVD vs. recovery in 65/77 patients, with raw accuracy of 84.4% (95% CI 74.4–91.7%). The model's raw sensitivity was 89.4% (95% CI 76.9–96.5%), and its raw specificity was 76.7% (95% CI 57.7–90.1%).

Conclusion: Fibrinogen plays a key role in the development of persistent defects after an acute thrombosis and can be used to predict recovery.

Disclosure of Interest: None declared.

PO477-MON

Residual thrombus mass and incidence of post thrombotic syndrome in patients treated with VKA or non-VKA oral anticoagulants in phase-III DVT treatment trials

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Background: Post-thrombotic syndrome (PTS) is a frequent complication of deep vein thrombosis (DVT) and 5–10% of patients develop severe PTS. Venous obstruction due to residual thrombus is a main risk factor for PTS development. Compared to vitamin-K-antagonists (VKA), non-VKA oral anticoagulants (NOACs) were found similarly effective to prevent recurrent DVT. However, little is known about the reduction of clot burden and the impact on PTS rates of NOACs compared to VKA.

Aims: To evaluate PTS incidence of PTS in long-term follow up after DVT therapy with NOAC or VKA.

Methods: During 2014, all patients treated for acute DVT in previous phase-III NOAC trials at our site were invited for late follow-up including assessment of PTS and residual clot burden, quantified with an ultrasound clot burden score (CBS).

Results: 229 of our 305 NOAC trial patients (75.1%) were available for follow-up. Baseline characteristics were similar for 113 NOAC and 116 VKA patients. Mean duration of anticoagulation was 8.2 ± 3.6 months and mean duration between DVT diagnosis and follow-up was 38.8 ± 15.9 months, during which 26 patients (11.4%) developed recurrent VTE.

Mean clot burden score at DVT diagnosis was 16.0 ± 8.6 in NOAC and 17.6 ± 8.9 in VKA patients ($P = 0.15$) and, at follow-up, decreased to 4.2 ± 4.8 and 4.6 ± 5.3 , respectively ($P = 0.48$).

At follow-up, mean Villalta score was 4.1 ± 2.8 and similar for NOAC (3.9 ± 2.5) and VKA (4.2 ± 3.1 ; $P = 0.49$). A Villalta-Score ≥ 5 (represents PTS) was found in 33.6% of NOAC and 39.7% of VKA patients ($P = 0.456$). A Villalta score of 10–14 (moderate PTS) was found in 1.8% of NOAC and 6.9% of VKA patients ($P = 0.12$). No venous ulcers were observed.

Conclusion: 30 to 40% of patients develop PTS after VTE with moderate PTS occurring in 2 to 7%. While clot burden at diagnosis was similar at baseline and demonstrated similar reduction during follow-up, rates of overall PTS and moderate PTS were numerically more com-

mon in VKA patients, which was not statistically significant, probably due to our limited cohort size.

Disclosure of Interest: None declared.

PO478-MON

The role of inflammation in post-thrombotic syndrome after pregnancy-related venous thrombosis

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Background: Studies have suggested that inflammation may play a role in the pathophysiology of the post-thrombotic syndrome (PTS).

Aims: To investigate whether patients with PTS after pregnancy-related deep vein thrombosis (DVT) have elevated inflammatory markers.

Methods: The present study included 313 women with pregnancy-related venous thrombosis during 1990–2003 and 353 controls. All women answered a comprehensive questionnaire and donated a single blood sample in 2006. In this sub-study only cases with DVT in a lower limb were included, whereas women, who were pregnant or were using anticoagulants or oral contraceptives at the time of blood sampling, were excluded. The final study population comprised 182 women with pregnancy-related DVT. PTS was diagnosed when a self-reported Villalta score was >4 , moderate/severe PTS when the score was >9 . As possible predictors of PTS or moderate to severe PTS we included the inflammatory markers high sensitivity CRP, interleukin (IL)-6, IL-8, IL-10, monocyte chemoattractant protein (MCP)-1, and the two adhesion molecules intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1.

Results: 41% (95% CI 34–48) of cases with pregnancy-related DVT in the lower limb were diagnosed with PTS 3–16 years after index pregnancy; 19% (95% CI 13–25) had moderate or severe PTS. In univariate analysis using >90 th percentiles among controls as cut-offs, only IL-10 was significantly associated with PTS (OR 10.8, 95% CI 1.3–89.8, $P = 0.009$), but not when adjusting for postnatal postpartum DVT, age, and regular smoking before pregnancy, which have previously been found to be independently associated with PTS in this population. Moderate to severe PTS were also not associated with the inflammatory markers.

Conclusion: Women with a history of pregnancy-related DVT who developed PTS did not have elevated inflammatory markers consistent with no or minimal systemic inflammation.

Disclosure of Interest: None declared.

PO479-MON

Mom, why should I wear these socks? Taking the first step in the journey to understanding the role of compression garments in the treatment of pediatric post-thrombotic syndrome

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Background: Elastic compression stockings/sleeves (ECS) are the cornerstone of treatment of post-thrombotic syndrome (PTS). Research in adults has found non-compliance rates as high as 63%, highlighting its importance in intervention-efficacy research. Little is known about the use of ECS in pediatrics.

Aims: To explore barriers in the compliance to ECS in children with PTS as a basis for improving the efficacy of this treatment.

Methods: Following a phenomenological design to qualitative research, children with a PTS diagnosis who had been prescribed ECS were enrolled until reaching data saturation. Patients/parents' interviews were semi-structured; the dyad approach was used to interview younger patients. Also, PTS symptoms and functional impact were assessed [PTS symptoms questionnaire and the Pediatric Outcome Data Collection Instrument (PODCI)]. Three compliance levels were defined: C1 Full, ECS use as instructed; C2 Partial, ECS use not as prescribed; and C3 None, no ECS use. Ethics approval and informed consents were obtained.

Results: 17 patients with a median (IQR) age of 15 (7) yrs, 5 (4) PTS symptoms, and global PODCI score of 88 (11) were enrolled. Compliance distribution was: C1 40%/C2 30%/C3 30%. Six barriers were identified: lack of patient understanding of their condition (47% of patients); ECS discomfort (47%); ECS appearance (41%); difficulty to apply ECS (41%); no perceived benefits (35%); cost (18%); cultural factors (6%). Interestingly, 2 facilitators were also established: clinician knowledge transfer (CKT) and access to specialized ECS care, which enhanced the patients' ability to overcome all barriers, except cost and cultural factors. Effective CKT strongly influenced compliance levels (C1 100%/C2 40%/C3 0% $P = .001$).

Conclusion: The described barriers and facilitators to ECS compliance provide a framework to understand and improve the use and efficacy of the only known PTS treatment. The next step is a trial of smart compression garments for an in-depth analysis of this framework.

Disclosure of Interest: None declared.

PO480-MON

Inadequate anticoagulation is the only risk factor for development of post thrombotic syndrome in unprovoked deep vein thrombosis

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Background: Post thrombotic syndrome (PTS) is a cause for morbidity in patients suffering from deep vein thrombosis (DVT).

Aims: To identify risk factors for PTS in patients with unprovoked DVT.

Methods: We conducted a prospective study of patients presenting with unprovoked lower limb DVT. Patients with secondary acquired causes such as surgery, malignancy were excluded. Patients wore compression elastic stockings below knee from early morning to bed-time for prevention of PTS. PTS was analysed and graded as per the Villalta PTS scale. Patients received anticoagulation and monitored as per standard guidelines. Adequate anticoagulation was assessed by time to anticoagulation (>1 month taken as inadequate) and/or INR of 2–3 on atleast 50% of visits. A prothrombotic work up for inheritable and immunological causes was done as part of this study. We also studied SNP mutations for tissue factor (TF 603A/G, TF 5466A/G) and tissue factor pathway inhibitor (TFPI 287T/C, –536C/T, –399C/T, –33T/C).

Results: 77 patients were enrolled (60males, 17 females); median age 30 years (range 16 years–65 years); median time to anticoagulation 30 days (1 day–10 years). 44(57%) received adequate anticoagulation. PTS was noted in 50(64.9%). An inheritable or acquired prothrombotic state was noted in 12 (8 had hyperhomocysteinemia, 3 had low protein C, 2 had low protein S, 1 patient had factor V Leiden mutation; 1 patient each had anti β_2 -GPI and DsDNA; 4 patients had >1 defect). Four patients with pre-existing thrombophilic state had PTS despite adequate anticoagulation and no other risk factors; 2 were inadequately anticoagulated and 1 was a chronic smoker. The remaining 5 had no PTS. 19 patients in the adequately anticoagulated group developed PTS (43%) as compared to 31/33 in the inadequately treated group (94%); $P < 0.0001$. None had a TFPI 536 C/T mutation. However, of the remaining 5 mutations studied, all patients had one mutation or other.

Conclusion: Inadequate anticoagulation was the only variable contributing to development of PTS.

Disclosure of Interest: None declared.

PO481-MON

Patient compression hosiery concordance

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Background: The Belfast Trust Venous thromboembolism (VTE) Clinic manages the treatment and review of outpatients diagnosed with deep vein thrombosis (DVT), pulmonary embolism (PE) and superficial thrombophlebitis (STP). In keeping with NICE clinical guidelines 144 (2012), the VTE clinic offers below –knee graduated compression hosiery. The advice is given to the patients to continue wearing the stocking(s) for at least two years, with the garment(s) to be replaced according to manufacturer's instructions.

Aims: To review all cases of DVT presenting for outpatient management and assess whether a repeat prescription of compression hosiery was provided by their primary care physician

Methods: Using the electronic care record (ECR) the team were able to determine if the patients who attended during 2013, were prescribed compression hosiery as requested by the VTE clinic and if the patients were issued a repeat prescription.

Results: Of 189 outpatients attending the VTE clinic, 117 had a DVT. Compression was contraindicated in 14, 2 patients refused to wear. 8 patients were lost to follow-up, including 4 deceased, 3 not resident in N.I and 1 patient whose demographics were not matching on ECR. In total 93 patients were reviewed electronically. 48 patients had prescriptions issued initially. Only 25 patients were recorded on ECR as having compression as a repeat prescription.

Conclusion: We were able to conclude, that in general, focus of VTE management is on anticoagulation, with little appreciation or understanding of the impact of post thrombotic syndrome. As result of our findings, we have incorporated a number of measures, to provide a more seamless transfer of care and improve both professional and patient awareness and concordance.

Disclosure of Interest: None declared.

Rare bleeding disorders I

PO482-MON

The utility of thrombelastography and multiplate as screening tools for rare inherited bleeding disorders not associated with a coagulation factor deficiency

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Background: Patients with inherited platelet disorders usually have symptoms of a primary hemostasis defect and a family history of bleeding. Thrombelastography (TEG[®]) is a whole blood coagulation analysis used to identify coagulopathy and guide transfusion in trauma and surgery. Impedance platelet aggregometry (Multiplate[®]) is validated to evaluate the effect of antiplatelet drugs. These bedside analyses are increasingly being used as preoperative screening tools.

Aims: The aim was to evaluate the sensitivity of TEG[®] and Multiplate[®] as screening tools for inherited defects in primary hemostasis

Methods: Consecutive patients referred for bleeding tendency and without evidence of a coagulation factor deficiency were evaluated. A standardized bleeding assessment tool (ISTH-BAT) score was performed. TEG parameters (reaction time (R), angle (A), maximum amplitude (MA), and lysis after 30 min. (LY30) were measured within 2 h of blood collection. Platelet function was evaluated by a Multiplate[®] analyzer following agonist stimulation with ADP, Arachidate, TRAP and Ristocetin.

Results: Fifty-seven patients (median age 40, range 19–75) with a median BAT score 10 (range 4–30) were included. A family history of bleeding was positive in 47/57 (82%) patients. Standard coagulation tests (INR, APTT, fibrinogen, VWF, VWFRIST and platelet count) were in the normal range. Abnormal bleeding during previous surgery was reported in 43/57 (75%). Normal results of TEG and Multiplate were found in 39/55 (71%) and 44/57 (77%), respectively. In 30/55 (55%) patients both TEG and Multiplate parameters were within the normal range.

Conclusion: In a group of patients with normal standard coagulation and vWF tests, TEG[®] and Multiplate[®] identified abnormality only in 45% despite increased risk of bleeding according to the BAT score. In patients with mild bleeding tendency, TEG[®], Multiplate[®] and standard coagulation and vWF tests seems to miss patients with clinical bleeding tendency. Further research is needed to identify these patients.

Disclosure of Interest: None declared.

PO483-MON

Bleeding score and clotting screening test performed before elective surgery to diagnose unknown hemorrhagic diseases

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Background: Congenital Hemorrhagic diseases (CHD) are often complicated by intra and post surgical bleeding, requiring diagnostic procedures. Strategies to evaluate the risk of hemorrhage are based on coagulation screening tests and bleeding history. Because of poor sensitivity and specificity of these strategies, it is difficult to distinguish between normal subjects and patients affected by mild bleeding disorders.

Aims: To assess if coagulation screening tests together with bleeding score (evaluated by medical staff experienced on hemostasis) may be useful to identify patients with coagulopathy, not already diagnosed.

Methods: Monocentric observational cross-sectional study conducted on 1503 consecutive patients before elective surgery. Bleeding score and first level laboratory tests evaluation (PT, PTT, platelets count) were performed. Patients were classified as normal or cases (with bleeding score positivity and/or with laboratory test alterations). Cases underwent secondary tests evaluation in order to identify coagulation disorders. To verify bleeding risk during and after surgery a 1:1 randomized matched cohort substudy was performed.

Results: Among the 1503 patients enrolled, 149 were cases: 55 adults and 94 pediatrics. Considering all cases, BS positivity was found in 82 cases (62 were pediatrics), prolonged PT was found in 17 cases, prolonged PTT in 34 and platelets reduction in 31 (24 were adults). Among 149 cases, 31 underwent secondary laboratory tests and among them we identified 2 Willebrand disease, 2 FXII deficiency and 4 patients with antiphospholipid antibodies. Among the 149 cases, 141 underwent surgery (52 adults and 89 pediatrics). Matching randomly cases and controls we didn't find any difference in bleeding during and after surgery.

Conclusion: Our data do not support the hypothesis that mild CHD are under diagnosed in general population. Furthermore the cohort substudy didn't detect differences in surgery bleeding complications.

Disclosure of Interest: None declared.

PO484-MON

Efficacy of FGTW fibrinogen concentrate in the treatment of bleeding episodes and prevention of excessive bleeding during surgery in patients with congenital fibrinogen deficiency

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Background: FGTW is a new generation of plasma-derived human fibrinogen concentrate recovered from the supernatant fraction of cryoprecipitate. Manufacturing process includes three specific viral inactivation and/or removal steps: solvent detergent treatment, nanofiltration and dry-heat treatment.

Aims: The aim of this multinational, multicenter, open-label, single arm, prospective study was to evaluate the efficacy and safety of FGTW in treatment of nonsurgical bleeding and prevention of excessive bleeding during surgery in subjects with congenital afibrinogenemia (antigen ≤ 0.2 g/L) or hypofibrinogenemia (antigen ≤ 0.5 g/L).

Methods: The study was approved by Ethics Committees. All subjects signed an informed consent. Dosing of FGTW was individualized to achieve peak fibrinogen of 1.2 g/L for major bleeding/surgery and 1.0 g/L for minor bleeding/surgery. The primary efficacy endpoint was investigator's overall assessment of hemostasis on the day of hospital discharge or at 24–48 hrs after treatment when not hospitalized. A 4-point scale (excellent, good, moderate, none) adapted to the clinical situation was used.

Results: Sixteen subjects (15 with afibrinogenemia), median age 18.5 yrs (range 7–37) participated in the study. Fifteen underwent 38 surgical procedures (10 major, 28 minor). All had an overall hemostatic efficacy rated 'Excellent'. Nine subjects experienced 32 nonsurgical bleeding events (23 major, 9 minor). Efficacy was rated 'Excellent' (29) or 'Good' (3). Median FGTW infusion dose (IV) was 0.055 g/kg (pre-surgical) and 0.053 g/kg (bleeding). Median fibrinogen level 30 min post-infusion was 1.28 g/L (surgery) and 0.98 g/L (bleeding), with normalization of global coagulation tests. Most events (65/70, 92.8%) required a single infusion of FGTW. There were 2 product-related SAEs (asymptomatic venous thrombosis).

Conclusion: This study showed that FGTW provided adequate hemostasis for the management of surgery and bleeding in subjects with afibrinogenemia, exhibiting a favourable benefit/risk ratio.

Disclosure of Interest: C. Djambas Khayat Grant/Research Support from: LFB Biotechnologies, Les Ulis, France, M. El Khorassani Grant/Research Support from: LFB Biotechnologies, Les Ulis, France, V. Gay Grant/Research Support from: LFB Biotechnologies, Les Ulis, France, M. Barthez-Toullec Employee of: LFB Biotechnologies, Les Ulis, France, C. Henriot Employee of: LFB Biotechnologies, Les Ulis, France, F. Bridey Employee of: LFB Biotechnologies, Les Ulis, France, L. Carreras Consultant for: LFB Biotechnologies, Les Ulis, France, C. Négrier Consultant for: LFB Biotechnologies, Les Ulis, France, P. de Moerloose Consultant for: LFB Biotechnologies, Les Ulis, France

PO485-MON

A new Swiss family with constitutional hypofibrinogenemia and liver disease

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Background: Fibrinogen storage disease (FSD) is characterized by hypofibrinogenemia and hepatic inclusions due to impaired release of mutant fibrinogen which accumulates and aggregates in the hepatocellular endoplasmic reticulum.

Aims: We studied a new Swiss family with fibrinogen Aguadilla. In order to understand the molecular particularity of FSD mutations, fibrinogen Aguadilla and the three other causative mutations, all located in the gD domain, were modelled.

Methods: The proband is a young Swiss girl investigated because of fatigue and elevated liver enzymes. Protein structure models were prepared using the Swiss-PdbViewer, the POV-Ray software and the 3fibA PDB file.

Results: The proband was found to be heterozygous for fibrinogen Aguadilla: FGG Arg375Trp. Laboratory analyses were unremarkable except for a prolonged INR (1.5) with low functional fibrinogen [Clauss 0.7 g L⁻¹ (ref. 1.8–3.6)] and raised liver enzymes [ASAT 111 U L⁻¹ (ref. <35), ALAT 125 U L⁻¹ (ref. <35)]. There was no history of bleeding. Familial screening revealed that her mother and maternal grandmother are also affected and in addition are respectively heterozygous and homozygous for the hereditary hemochromatosis mutation HFE C282Y. Models of backbone and side-chain interactions for fibrinogen Aguadilla in a 10-angstrom region revealed the loss of five H-bonds and the gain of one H-bond between structurally important amino acids. The structure predicted for fibrinogen Angers showed a novel helical structure in place of hole 'a' on the outer edge of gD likely to negatively impact fibrinogen assembly and secretion.

Conclusion: The mechanism by which FSD mutations generate hepatic intracellular inclusions is still not clearly established although the promotion of aberrant intermolecular strand insertions is emerging as a likely cause. Reporting new cases is essential in the light of novel opportunities of treatment offered by increasing knowledge of the degradation pathway and autophagy

Disclosure of Interest: None declared.

PO486-MON

Safe use of FXI concentrate in a single centre experience over 19 years

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Background: Factor XI (FXI) concentrate is a human plasma-derived substitute for fresh frozen plasma to provide patients with FXI deficiency with more consistent haemostatic cover in emergency or elective situations. It has previously been implicated in adverse events such as thrombosis and inhibitor formation, with rare case reports of fatal incidents.

Aims: In our retrospective case series we sought to establish the incidence of such complications through our single centre experience in a Haemophilia Comprehensive Care Centre at Royal Free Hospital, London, UK between 1994 and 2012 inclusive.

Methods: Patients who received FXI concentrate had their medical records analysed relating to age, baseline FXI level and indication of concentrate. Specific adverse events such as failure of treatment with further bleeding, or other events including suspected viral transfusion transmitted infection (TTI), inhibitor formation to FXI administration and thrombosis were recorded.

Results: Eighty-six patients received 242 treatment episodes of FXI concentrate within a 19 year period. 12 (5%) of all treatment episodes were related to an adverse event. Of the total 12 adverse events, 8 were related to persistent bleeding post concentrate infusion. No viral TTIs were identified. There were two recorded inhibitors, one thrombotic event and one transfusion reaction. No statistical differences were noted between age and an adverse event ($P = 0.357$), or between FXI dose per kilogram and subsequent bleeding ($P = 0.506$). Numbers of adverse events were too low to distinguish between bleeding between fibrinolytic and non-fibrinolytic sites in those who had a failure of treatment. None of the patients documented as having an adverse event suffered long term morbidity as a result.

Conclusion: Our experience of FXI concentrate use demonstrates infrequent minor adverse events related to its administration and is a safe product to use.

Disclosure of Interest: G. Ling: None Declared, H. Kagdi: None Declared, P. Chowdary Grant/Research Support from: Novo Nordisk, Pfizer, CSL Behring, Consultant for: Novo Nordisk, Pfizer, CSL Behring, Baxter, Biogen Idec, Speaker Bureau of: Bayer, Novo Nordisk, CSL Behring, Baxter, K. Gomez: None Declared

PO487-MON

The epidemiology of inherited bleeding disorders in Newfoundland and Labrador, Canada

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Background: Due to the pattern of settlement, Newfoundland and Labrador (NL) is characterized by a unique array of genetic inheritance. Traditionally, we have had an abundance of small outport communities and very large families, which have provided the conditions for a relatively large pool of patients with hereditary bleeding disorders (HBD). However, most recent population fertility rates of NL was 1.24 in 2011, hence the opportunity to study large families with rare disorders is diminishing.

Aims: Our goal is to document our unique pattern of inheritance as the basis for ongoing research.

Methods: We validated the diagnostic laboratory results from our HBD registry developed in 1988. We compared our pattern of HBD with those reported by the World Federation for Hemophilia in 2012 and the Canadian Hemophilia Registry in 2013 to contrast prevalence of von Willibrand's disease, Hemophilia A & Hemophilia B as well as Factor deficiencies I, IV, VII, X, XI and XIII. Beyond this, we also documented all the available genetic mutations associated with each HBD.

Results: Interestingly, we have 2.86 and 10.9 times the prevalence of Hemophilia A recorded than mainland Canada and Worldwide respectively. The most striking data is the per capita prevalence of Factors I, II, V, XI, and XIII which are higher than that of Canada by a factor of 15.45, 5.12, 5.63, 5.39, 5.31 respectively and the world by a factor of 204, 51.2, 39.2, 25.7, 68.2 respectively. Hemophilia A patients were predominantly Val 2016 Ala mutation and FXIII patients all inherited the c691-1 G>A genotype.

Conclusion: These results show that NL unique geography and population distribution has that has increased per capita prevalence of some rare factor deficiencies by genetic drift. This prevalence establishes NL distinctively in terms of genetic variation, ideal for further research. However, with an aging population, and less average births per family, this population is in rapid decline and thus provides a limited window of opportunity for research.

Disclosure of Interest: None declared.

PO488-MON

Pharmacokinetic and pharmacodynamic results of a multicenter, open-label, single arm study with a fibrinogen concentrate (FGTW) in subjects with afibrinogenemia

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Background: Bleeding in congenital fibrinogen deficiency is commonly treated with cryoprecipitate, fresh frozen plasma, and when available, fibrinogen concentrates. LFB has developed a new generation of human plasma-derived fibrinogen concentrate (FGTW) recovered from the supernatant fraction of cryoprecipitate. Manufacturing process includes 3 biological safety steps.

Aims: The objective of the study was to assess the pharmacokinetics (PK) and pharmacodynamics (PD) of FGTW in afibrinogenemic subjects (antigen < 0.2 g L⁻¹) of more than 40 kg b.w.

Methods: PK/PD assessment was performed after a single infusion of 0.06 g kg⁻¹ of FGTW. Concentrations of fibrinogen expressed in antigen and activity as well as global coagulation tests (aPTT, PT, TT) and clot firmness (ROTEM) were followed during 14 days after infusion. PK parameters were estimated by non-compartmental analysis with Phoenix WinNonLin. Results are presented using geometric mean values. This multicenter study was approved by Ethics Committees. All subjects signed an informed consent.

Results: Fourteen subjects of 11 to 38 year-old, including 9 adults and 5 children, with bodyweight ranging from 44 to 93.5 kg, were administered FGTW over 43 to 120 min. A peak plasma activity of 1.41 g L⁻¹ (range 1.06–2.19) was reached at the first time point (1 h) post-dose, followed by a monoexponential decay with a terminal half-life of 69.3 h (range 51.0–99.9). Incremental recovery was 23.5 g L⁻¹ per g kg⁻¹ (range 17.7–36.5). Similar results were obtained for fibrinogen antigen. PK/PD relationships showed that normalization of the global coagulation tests and maximum clot firmness was obtained for concentration of fibrinogen activity between 0.5 and 1 g L⁻¹, depending on the test.

Conclusion: These results show that in subjects of more than 40 kg b.w, 1 mg kg⁻¹ increases fibrinogen level by 2.3 mg dL⁻¹ with a half-life of about 3 days. This provides a solid basis for the treatment with FGTW in patients with afibrinogenemia.

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PO489-MON

Alternative strategy to treat hemophilia trough protein S inactivation: insight from a mouse model

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Background: Protein S (PS) is an anticoagulant that limits thrombin generation, acting as a cofactor of APC and TFPI. Human homozygous PS deficiency leads to disseminated intravascular coagulation (DIC) while heterozygous deficiency increases risk of thromboembolic events. Although mouse models recapitulate aspects of PS deficiency in human, no alive PS^{-/-} mice have been observed: they die at late pregnancy due to consumptive coagulopathy.

Aims: To improve the impaired clotting potential caused by FVIII (HA) or FIX (HB) deficiency we investigated the effect of PS inhibition in mice.

Methods: Mouse matings were set to generate HA-PS^{-/-} and HB-PS^{-/-}. Offspring was checked for possible overt DIC then *in vivo* investigated with models of venous thrombosis (VTE), tail clipping (TC) and acute hemarthrosis (AH).

Results: HA and HB rescued the lethal PS^{-/-} embryonic phenotype: HA-PS^{-/-} and HB-PS^{-/-} pups were found alive, at Mendelian frequency (80/366, 22% and 17/94, 18% respectively) and developed normally. These mice confirmed undetectable plasma coagulant FVIII/FIX (HA/HB) and antigenic PS levels. Blood parameters investigation did not show relevant DIC signs: platelets number, TAT and fibrinogen levels were comparable to HA/HB mice. Once challenged in TF driven VTE HA-PS^{-/-} mice did not show altered mortality compared to HA mice ($n = 15$, 88% vs. $n = 19$, 100% respectively). In the TB, HA-PS^{-/-} mice bled less (158 ± 11 uL $n = 6$) compared to HA and HA-PS^{+/-} (247 ± 19 uL, $n = 10$ and 239 ± 11 uL, $n = 8$ respectively, $P < 0.006$). In AH, joint swelling was less prominent (0.02 ± 0.01 vs 1.054 ± 0.29 in HA, $n = 5$ $P = 0.01$ at 24 h) and stable in HA-PS^{-/-} than HA mice (0.02 ± 0.07 vs 0.92 ± 0.17 , $n = 7$ $P = 0.002$ at 72 h).

Conclusion: Lack of PS in hemophilic mice does not increase thrombotic risk nor leads to DIC meanwhile reducing significantly hemophilia A bleeding and limiting trauma-induced joint swelling. These preclinical data highlight PS as new potential target in hemophilia therapy.

Disclosure of Interest: None declared.

PO490-MON

Genotype and phenotype report on patients with congenital fibrinogen deficiency in south of Iran

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Background: Fibrinogen has a pivot role in coagulation pathway. Congenital afibrinogenemia is a rare bleeding disorder which is inherited as homozygote or compound heterozygote to become symptomatic.

Patients with congenital fibrinogen deficiency may present with hemorrhagic or thromboembolic clinical signs.

Aims: We aimed to evaluate genotype and phenotype findings in patients with congenital fibrinogen deficiency in Southern Iran.

Methods: Genotype and phenotype of 18 patients of 18 unrelated families which congenital fibrinogen deficiency was diagnosed for them at Shiraz Hemophilia Center were investigated. Mutated gene in affected individuals was detected by DNA sequencing. Also factor level and clinical sign presentation at the onset of diagnosis was studied for each patient.

Results: We found nine different mutations that three of them were novel. The most common clinical sign on diagnosis were ecchymosis - purpura (38/9%) and umbilical cord bleeding (22/2%). The mean of age at onset was 25.86 ± 26.78 months (median: 18 months). Mean factor coagulant level was 48.5 ± 37.32 mg dL⁻¹ (median: 70 mg dL⁻¹). Two death patients presented with severe bleeding including hemarthrosis and intra cranial hemorrhage (ICH) in one patient (factor activity level <75 mg dL⁻¹), and post partum pulmonary emboli in the other one (factor activity level <35 mg dL⁻¹). The underlying mutation was only determined in first patient: g.1787_1789delGTC and 1787ins12 bp. There was no statistically significant differences between males and females with regard to age at diagnosis or factor level ($P = 0.246$ and 0.596 respectively).

Conclusion: Based on our results, g.1787_1789delGTC and 1787ins12 bp has been associated with severe bleeding including hemarthrosis and ICH in congenital fibrinogen deficiency. It seems that direct relationship between phenotype and severity of factor deficiency cannot be found at least in some cases.

Disclosure of Interest: None declared.

PO491-MON

FVIIIa mimicking bispecific antibody, ACE910 improves *in vitro* coagulation function of fxi deficiency patient's plasma

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Background: Factor (F)XI deficiency presents marked prolongation of aPTT, and the bleeding symptom is not rare, which are milder than hemophilia A. Fresh frozen plasma and/or plasma-derived FXI concentrates are used for haemostatic therapy. ACE910, recombinant bispecific monoclonal antibodies to FIXa and FX, mimics the FVIII cofactor function, and improves aPTT and thrombin generation in FVIII-deficient plasma by substituting for FVIII without activation phase.

Aims: We examined the effects of ACE910 on FXI-deficient plasma to assess the further application of its antibody for FXI deficiency.

Methods: Commercial FXI-deficient plasmas (George King, $n = 11$) in the presence of ACE910 ($0-100 \mu\text{g mL}^{-1}$) were examined by clot waveform analysis (CWA) and thrombin generation assay (TGA). aPTT reagent (aPTT-trigger) and the mixture of ellagic acid and tissue factor (mixed-trigger) were used as the trigger for these assays. Clot time (CT) and maximum coagulation velocity (|minI|) in CWA and peak height in TGA were evaluated.

Results: Coagulation factor activity (FVIII, FIX, FX and FXI) of all samples were $125 \pm 29\%$, $111 \pm 32\%$, $92.4 \pm 12.7\%$, and $<1\%$, respectively. In CWA, CT and |minI| of normal pooled plasmas observed by aPTT-trigger and mixed trigger was $34.5/98.6$ sec and $2.98/0.80$, respectively. The presence of ACE910 shortened the CT of all FXI-deficient samples in both aPTT-trigger and mixed-trigger (93.2 ± 6.9 to 40.7 ± 2.6 sec and 223 ± 19 to 209 ± 18 sec, respectively) in dose-dependent manners, and |minI| was similarly improved (39.6 to 56.0% of normal and 35.0 to 41.3% , respectively). In the TGA, peak height of 7 samples was improved by $\sim 40\%$ of normal by

mixed-trigger, although the thrombin generation was any little detected in all samples by aPTT-trigger.

Conclusion: ACE910 improves the potentials of coagulation function of FXI-deficient plasma as well as FVIII-deficient plasma. This result suggests the possibilities for the clinical application of ACE910 in FXI deficiency.

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PO492-MON

Spectrum of factor XI mutations in the Chinese population

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Background: Prevalent in people of Jewish ancestry, congenital Factor XI (FXI) deficiency is an autosomal recessive bleeding disorder, yet statistics of morbidity rate in other races is not clear currently. Up to 220 mutations have been found till now according to the FXI mutation database. Interestingly, considerable genetic heterogeneity occurs in various ethnic groups.

Aims: To study the spectrum of *F11* gene mutations in China by studying 53 unrelated Chinese patients diagnosed with FXI deficiency.

Methods: Following the direct sequencing of fifteen exons of *F11* gene, mutations were identified. Heterozygous individuals with low activity level were tested by the AccuCopy technique, a copy number variation genotyping method to explain for the contradiction between phenotype and genotype. A mRNA transcript was also characterized by RT-PCR to carry out the splicing mechanism of a specific splice site mutation.

Results: A total number of 38 mutations were identified in 53 patients. Of all the participants, 15 are homozygotes, 30 are compound heterozygous individuals and 8 are heterozygous carriers. These 38 different sequence variants includes 19 missense mutations, 9 nonsense mutations, 7 splice site mutations and 3 small deletions. CNV measurement, failed to get positive results. W228* (16 cases, 15.1%) turned out to be the most frequent mutation, followed by G400V (13 cases, 12.3%), c.1136-4delGTTG (11 cases, 10.4%) and Q263* (10 cases, 9.4%). The pathogenesis of c.1136-4delGTTG was confirmed by RT-PCR.

Conclusion: Defects in *F11* gene result in FXI deficiency with worldwide distribution and it remains a heterogeneous disease at the molecular level. W228*, G400V, c.1136-4delGTTG and Q263*, adding up to 47.17%, have turned to be the most four common mutations in Chinese FXI deficiency patients, which is quite different from the mutation spectrum of other populations earlier established.

Disclosure of Interest: None declared.

PO493-MON

Characterizing mechanisms of factor VIII binding to the low-density lipoprotein receptor-related protein 1

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Background: Hemophilia A is a bleeding disorder caused by a deficiency in coagulation factor VIII (fVIII). Current prophylactic replacement therapy, while effective, is difficult to maintain due to cost and frequency of injections. Hepatic clearance of fVIII is mediated by LRP1, a member of the LDL receptor family. The canonical model of ligand binding to these receptors involves lysine residues on the ligand docking in acidic pockets on the receptor. However, studies thus far have not been able to identify critical lysine residues in fVIII necessary for binding, suggesting that fVIII may bind to LRP1 via a unique mechanism.

Aims: While it is well established that fVIII binds LRP1, the molecular details of this interaction are unclear. Our goal was to define the molecular interactions between fVIII and LRP1 by investigating the ionic strength dependence of binding and by identifying LRP1 regions responsible for this interaction.

Methods: The effect of ionic strength on the binding affinity of fVIII to LRP1 was measured by surface plasmon resonance and ELISA.

Results: Debye-Hückel plots of ionic strength vs logK_D revealed a slope of 3.0, revealing that three ionic interactions contribute to binding. Thus the fVIII/LRP1 interaction fits the canonical model despite previous unsuccessful attempts at lysine mutagenesis. Furthermore, we mapped the binding of fVIII to the Cluster II and IV regions of LRP1. Finally, since full-length (FL) fVIII and B-domain deleted (BDD) fVIII are both used in replacement therapy, we confirmed that both forms of fVIII bind LRP1 with similar high affinity.

Conclusion: Our data suggests that fVIII interacts with LRP1 via the canonical model with three ionic interactions contributing to binding. Further, removal of the B-domain does not significantly alter the interaction of fVIII with LRP1. Together these data reveal important insights into the binding between fVIII and LRP1 and will help us to investigate ways to extend fVIII half-life *in vivo* to improve prophylactic replacement therapy.

Disclosure of Interest: None declared.

PO494-MON

Rare bleeding disorders: 17 years of diagnosis in the hemostasis laboratory of the hemostasis laboratory of the department of hematology of the university hospital of beni messous, algiers, algeria

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Background: Algerai is an emerging country of North Africa where rare bleeding disorders (RBDs) increases because consanguineous marriages are still customary.

Aims: The aim of our study is to appreciate the frequency of RBDs, the severity of the factor deficiencies & the clinical manifestations in the patients we diagnosed.

Methods: We tested patients with bleeding disorders &/or abnormal hemostasis tests, we realized PT/APTT & specific dosages of clotting factors. Patients were tested at least twice on different blood samples. we eliminated acquired hemostasis anomalies. Our laboratory partici-

pates to an international external quality assessment program. Clinical symptoms & histories were registered for the patients & their relatives & family trees were realized.

Results: In 17 years of activity, 322 patients (range age from new born to 86 years) were diagnosed: 19 FI, 06 FII, 68 FV, 163 FVII, 34 FX, 01 FX associated with von Willebrand Disease type 1, 08 FXI, 12 FXII, 01 FXIII, 02 FV/FVII, 03 FV/FVIII, 04 FV/FX, 01 FVII/FX, from mild to severe. A positive bleeding history was found for 71 (22%) & more frequently for homozygous factor deficiencies. The most common sites of bleeding were skin & mucous membranes.

Conclusion: RBDs are surely under detected in Algeria because of variable nature of relatively mild symptoms & the lack of adequate hemostasis laboratory means. Education & genetic counseling are needed to prevent & limit the spread of such common occurrence of RBDs, moreover because factor concentrates are expensive & not available for some deficiency states.

Disclosure of Interest: None declared.

PO495-MON

The thrombin generation assay is superior to thromboelastography at determining bleeding phenotype in congenital FXI deficiency

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Background: In the absence of an effective test to predict bleeding risk, FXI deficient individuals may be over-treated with FXI replacement (risks of thrombosis or transfusion related complications) or under-treated (risk of haemorrhage). Global haemostasis assays can identify bleeding tendency in a variety of haemostatic disorders.

Aims: To investigate if thromboelastography (±tpa) and thrombin generation assays (TGA) can differentiate between controls and FXI deficient individuals and between bleeders and non-bleeders.

Methods: Thromboelastography (±tpa) in whole blood (WB) with corn trypsin inhibitor (CTI) and platelet rich plasma (PRP) with CTI, and TGA in PRP and platelet poor plasma (PPP) with and without CTI were measured over a range of tissue factor concentrations in controls (*n* = 50) and FXI deficient individuals (*n* = 97) (bleeder (*n* = 24), non-bleeder (*n* = 50) and indeterminate (*n* = 23) by bleeding history).

Results: Thromboelastography in WB with CTI and tpa-thromboelastography in all samples (TF 0.12pM) did not differentiate between controls and FXI deficient individuals, nor between bleeders and non-bleeders. Thromboelastography in PRP with CTI allowed differentiation between bleeder and non-bleeders: CFT (434 ± 179s vs 277 ± 70s) (mean ± sd) (*P* < 0.01) and α angle (43.8 ± 9.5° vs 52.4 ± 5.8°) (*P* < 0.001). ROC analysis (CFT AUC = 0.891, *P* = 0.0014) (α angle AUC = 0.7804, *P* = 0.006) showed low specificity of 38% at 100% sensitivity for both. TGA differentiated between controls and FXI deficient individuals using ETP and peak height at TF0.5pM in all sample types except PPP with CTI. TGA in PRP with CTI allowed best differentiation between bleeders and non-bleeders (ETP ROC AUC = 0.9362 *P* < 0.0001, peak height ROC AUC = 0.9362 *P* < 0.0001) with specificities of 80% (ETP) and 67% (peak height) at 100% sensitivity.

Conclusion: Both TGA and thromboelastography in PRP with CTI can differentiate between bleeders and non-bleeders in FXI deficiency but TGA has a superior test performance at determining bleeding phenotype.

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PO496-MON

A new mutation in the *svil* gene is associated with autosomal dominant familial gray platelet syndrome

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Background: Gray platelet syndrome (GPS) is an inherited platelet function disorder of insufficient alpha-granule function. GPS is characterized by mild bleeding and presence of agranular platelets in blood. Its molecular mechanisms are poorly understood, and it can be supposedly caused by mutations in either of three genes: NBEAL2, GATA1 or GFI1B.

Aims: Here we investigated a family that included four generations of patients with autosomal dominant GPS using functional platelet assays, full-exome sequencing, and molecular dynamics simulations.

Methods: The family included 10 members, of whom 5 has clinical symptoms of mild bleeding. Platelets were analyzed using microscopic smear examination, flow cytometry, and confocal microscopy. Full-exome sequencing of all family members was performed using Ion Proton System. To find the genomic loci inherited similarly to the disease inheritance pattern, we performed nonparametric linkage analysis with MERLIN software package. Molecular dynamics simulations employed the NAMD 2.9 software. Homology modelling was done using PSIPRED server with GenTHREADER tool and Modeller 9.14.

Results: GPS was diagnosed on the basis of smear examination and decreased P-selectin expression upon activation. Analysis of the 10 exomes of the family members revealed a single-nucleotide heterozygous substitution SVIL:NM_021738:exon8:c.C1223T:p.T408M in the 10th chromosome, which was the only relevant mutation shared by patients and absent in healthy relatives (with no such mutations in either NBEAL2, GATA1 or GFI1B). This gene encodes a protein supervillin that is known to mediate interactions between cytoskeleton and nuclear membrane. Sanger sequencing confirmed this mutation. Computer modeling of supervillin with a T408M substitution revealed a disruption of the native folding in the affected region.

Conclusion: A novel mutation associated with familial GPS is discovered in the gene encoding supervillin. This can shed light on the molecular mechanisms of platelet production and causes of GPS.

Disclosure of Interest: None declared.

PO497-MON

Acquired GPVI deficiency in two unrelated patients with mild thrombocytopenia

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Background: Rare acquired glycoprotein (GP) VI deficiency is usually associated with immune/tumoral diseases, variable extent of thrombocytopenia and a bleeding diathesis.

Aims: To investigate two unrelated patients with GPVI abnormalities and moderate thrombocytopenia but no underlying disease.

Methods: Platelet count, function, glycoprotein expression and antiplatelet antibodies were explored in both patients.

Results: Case 1 is a 37y female with lifelong moderate thrombocytopenia and mild bleeding diathesis, but no other analytical abnormalities. Repeated sampling across 5y consistently showed spontaneous, rapid and temperature-dependent platelet clumping in tubes containing citrate or heparin but not EDTA or anti- α Ib β 3 antibody. Her platelets showed elevated P-selectin, CD63 and α Ib β 3 expression, TxA₂ production, and reduced levels of GPIb α and GPVI. Soluble GPVI was 3-fold higher than in healthy donors. Her plasma activated allogeneic platelets except platelets from a GPVI-deficient individual. This was blocked selectively by Src/Syk inhibitors, soluble GPVI-Fc fusion protein, or by immunodepletion of IgM from patient serum, consistent with an anti-GPVI IgM. Case 2, 37y female, had 1y history of thrombocytopenia, menorrhagia and spontaneous ecchymoses, commencing few months after unremarkable childbirth. She showed abnormal PFA-100 closure times and severe aggregation defect to epinephrine, collagen, collagen-related peptide and convulxin. Aggregations with other agonists were normal. Her platelets showed normal expression of α Ib β 3, GPIb/IX and α 2 β 1, but deficiency (~20%) of GPVI. No antiplatelet autoantibodies were detected. Prednisone treatment (1 mg kg⁻¹ day⁻¹, 1-month) normalized platelet count, GPVI expression and aggregation response. Thrombocytopenia and GPVI loss re-emerged on cessation of therapy.

Conclusion: Monitoring GPVI levels and presence of anti-GPVI autoantibodies may aid clinical evaluation of patients with acquired unexplained thrombocytopenia. PI14/01956, Br Heart Foundation, NHMRC Australia.

Disclosure of Interest: None declared.

PO498-MON

Identification of the pathophysiological mechanism of the VKCFD2 causing mutation P.ARG98TRP in human VKORC1 gene

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Background: Vitamin K Oxidoreductase Complex subunit 1 (VKORC1) is the key enzyme of the vitamin K cycle catalyzing the reduction of vitamin K and vitamin K 2,3-epoxide to vitamin K hydroquinone. This reduction is essential to sustain γ -carboxylation of the vitamin K dependent coagulation factors (e.g. FII, FVII, FIX, FX) by the enzyme γ -glutamyl carboxylase. Only one mutation in human VKORC1 is reported to cause reduced vitamin K oxidoreductase activity leading to combined deficiency of vitamin K dependent clotting factors type 2 (VKCFD2). The pathophysiological mechanism underlying this mutation is still unknown.

Aims: Aim of our study was to identify the molecular mechanism of VKORC1:p.R98W.

Methods: The sequence of hVKORC1 was analyzed *in silico* by different motif databases and revealed a putative di-Arginine ER retention motif in the region of R33_R35 and R98_R100. To confirm these results we cloned the cDNA of wt hVKORC1 into the pEGFP-N3 vector and generated following variants by site directed mutagenesis: R98W, R98A+R100A, delAR98-R100, and R33A+R35A. HEK293T cells were plated on cover glasses, transfected with all vectors, immunostained (ER, Golgi apparatus, plasma membrane, nucleus) and analyzed by confocal microscopy. Degree of co-localization for all variants was calculated using ImageJ 1.43 m software.

Results: All variants affecting R98_R100 show drastic decrease in ER co-localization compared to wt VKORC1, which is exclusively located

in the ER. The VKCFD2 causing mutation R98W results in 20% ER-localization only. The R98_R100 variants are not re-localizing to Golgi apparatus neither to the plasma membrane. The variant R33A+R35A affecting the second putative motif does not show any effect on ER retention.

Conclusion: Here, we identified a di-Arginine ER retention motif in hVKORC1 (R98_R100). The mutation p.R98W disrupts this motif and decreases the protein's ER retention capacity resulting in sub-cellular mislocalization, thereby causing the VKCFD2 phenotype.

Disclosure of Interest: None declared.

PO499-MON

Scott syndrome platelets show alterations in protein expression levels, calcium-dependent phosphorylation and cleavage as revealed by proteomics

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Background: The Scott syndrome is a rare bleeding disorder with a mutation in the transmembrane protein, anoctamin-6. Blood cells from Scott patients are impaired in Ca^{2+} -dependent phosphatidylserine exposure, ion conductance, membrane blebbing and microparticle formation.

Aims: We hypothesized that altered post-translational protein modifications are responsible for this complex phenotype.

Methods: Platelets from healthy control donors and a Scott patient were activated with the Ca^{2+} -mobilizing agents, convulxin/thrombin or ionomycin. Samples were used for advanced quantitative (phospho) proteomics and determination of neo-N-terminal protein cleavage sites.

Results: Quantitative proteomics analysis indicated 109 upregulated and 78 down regulated proteins in Scott platelets when compared to controls. Increased in the patient platelets were transcription factors; decreased were anoctamin-6, metabolic enzymes, Ca^{2+} -dependent proteases (calpain-2, presequence protease) and Ca^{2+} -binding proteins (S100-A9, grancalcin). Phosphorylation analysis indicated >2200 different phospho-sites which after thrombin stimulation overlapped (99.3%) between patient and control platelets. With Ca^{2+} -mobilizing agonists, the overlap reduced to 81%. Strongly activated Scott platelets displayed more frequently increased than decreased phosphorylation sites. The most altered phosphorylation proteins of Scott platelets were assigned to actin/tubulin cytoskeleton (28%), adhesion (22%), signaling/adaptor proteins (24%) and receptor-linked cytoskeleton (8%). Cleavage sites were detected of 818 proteins. In Scott platelets, diminished cleavage was found in the calpain substrates, caldesmon, calpain-1, talin-1, Src and VASP. Western blotting confirmed reduced calpain-mediated cleavage in the activated Scott platelets.

Conclusion: Advanced proteomic profiling provides novel insight into the altered protein machinery responsible for the major Ca^{2+} - and cytoskeleton-dependent membrane defects in Scott syndrome.

Disclosure of Interest: None declared.

PO500-MON

A failure to diagnose dysfibrinogenaemia: data from multicentre studies amongst UK Neqas and PRO-RBDD project laboratories

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Background: Mutations in the genes coding for fibrinogen (Fgn) causing dysfibrinogenaemia can result in either a bleeding or thrombophilic diathesis. However, these rare disorders are infrequently encountered in hospital laboratories, and the utility of different assays in diagnosis of dysfibrinogenaemia has not been previously explored in a multicentre study.

Aims: We describe here an exercise in which centres from the PRO-RBDD project (prospective data collection on patients with fibrinogen and Factor XIII deficiencies), and UK NEQAS centres performed investigations for dysfibrinogenaemia.

Methods: Three samples from donors with dysfibrinogenaemia (Sample 1: *gamma p.Arg301Cys*), sample 2 (*Bbeta166Arg3Cys* – Fgn Longmont), sample 3 (*Aalpha p.Arg35His*) and a normal donor were lyophilised and sent to UK NEQAS and PRO-RBDD centres, who were asked to perform investigations for possible dysfibrinogenaemia, and interpret their results. Median, coefficient of variation and range were determined for each assay method and reagent group.

Results: Results were returned from 62 UK NEQAS and 24 PRO-RBDD centres. PT, APTT, Clauss fibrinogen and thrombin times were performed by over 90% of centres, with 51% performing reptilase times, and 31% Fgn antigen. All centres providing interpretations identified samples 1 and 3 as abnormal. However, 39% of centres reported a normal or raised fibrinogen levels for the Fgn Longmont sample, and marked differences in Clauss fibrinogen results with different reagents were noted for this sample only (median 1.01 vs 5.10 g L⁻¹ for the two mostly widely used reagents).

Conclusion: Laboratories were successfully able to diagnose 2 dysfibrinogenaemia defects, but many centres failed to diagnose Fgn Longmont, and reagent discrepancies require further investigation.

Disclosure of Interest: None declared.

PO501-MON

Post authorization safety study of a triply secured fibrinogen concentrate in congenital afibrinogenemia. A prospective observational study

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Background: A new fibrinogen concentrate was developed according to European guidelines on plasma-derived products. Subsequently a post marketing safety study was conducted.

Aims: The main objective of this non-interventional, prospective, non-comparative, multicenter study was to evaluate the safety of this new marketed fibrinogen concentrate for the treatment of congenital fibrin-

ogen deficiency in current medical practice. The secondary objective was to assess its clinical efficacy.

Methods: A descriptive analysis was done according to the following criteria: prophylaxis or on-demand treatment and age (<6, <12 and ≥12 years).

Results: Fourteen afibrinogenemia patients (1–78 years) were included and followed for 1 year. Twenty-one adverse drug reactions (ADRs), classically reported with administration of fibrinogen concentrate (pallor, chills, cough, vomiting, headache, urticaria and erythematous rash), were reported in 5/14 patients; 14 ADRs occurred in 2 children under 6 years and 7 ADRs concerned 3 adults. There were 2 serious ADRs, an anaphylactic shock in a 22-month-old child and a subclavian venous thrombosis in a 35-year-old woman with an implantable port, both treated with prophylaxis. The outcome of the 2 serious ADRs was favorable without sequelae; no patient had to stop definitively the treatment because of an ADR. In 9 patients under prophylaxis, 365/367 (99.5%) infusions were considered successful (excellent/good) and 2 as failures (moderate). Moreover, 10 mainly trauma-related hemorrhagic situations in 4 patients required 16 curative infusions. For the 5 patients treated on-demand, the efficacy was rated as excellent for 27/48 infusions and good for the 21 others.

Conclusion: This study confirms that the benefit/risk ratio of this fibrinogen concentrate is favorable. The diversity of the patients included in terms of age, sex, comorbidity and type of treatment provides valuable information on current delivery of medical care for afibrinogenemia patients.

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PO502-MON

Fibrinogen deficiency: preliminary results of the PRO-RBDD project

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Background: Management of fibrinogen deficiency is challenging and guidelines for optimal treatment are not available yet.

Aims: The 3-year observational PRO-RBDD study aims at evaluating the incidence of bleeding episodes in patients with fibrinogen deficiency, the association between fibrinogen activity and bleeding severity and the used treatment regimen.

Methods: Data were entered in a web-based database at baseline (history) and every 6 months (follow up). 114 patients (66 females/48 males; 31 patients ≤12 years) were recorded.

Results: Regression analysis confirmed a good association between fibrinogen activity and age at diagnosis or clinical severity (classification in Peyvandi et al JTH 2012) ($\beta = -0.19$, $P < 0.05$). Grade III bleedings were observed in 67% (18/27) of patients with undetectable fibrinogen activity, however only 38% of these patients were on prophylaxis. The most frequent symptoms were mucocutaneous bleedings, hematoma and hemarthrosis (40, 23 and 21%) observed in all laboratory severities; while CNS, GI and umbilical cord bleeding were observed only in severe and moderate deficiency (8, 4 and 15%). Almost 30% (13/45) of women had menorrhagia; 15% (6/40) of pregnancies ended to a spontaneous abortion and 8% (3) showed bleeding complications. Follow-up data (up to 700 days) on 75 patients on 'on-demand' therapy and 12 on prophylaxis, using 50–670 mg Kg⁻¹ month⁻¹, showed an incidence bleeding rate of 0.3 patient-year⁻¹ (95%CI 0.2–0.5) and 0.5 (95%CI 0.2–1.1). The risk of having a bleeding requiring replacement therapy did not differ in the two groups (log rank test = 0.43). No thrombotic events were observed.

Conclusion: These results confirmed a good association between fibrinogen activity and clinical severity. A wide range of prophylaxis dosage were used without a complete resolution of bleeding episodes underlying

ing the lack of indications for optimal prophylactic treatment. A larger group of patients and a longer follow up period are required to evaluate the efficacy of prophylaxis.

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PO503-MON

Decreased fibrinolytic resistance in FXI-deficiency is independent of clotting impairment

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Background: Factor XI deficiency (FXI-Def) is a rare bleeding disorder with no association between the circulating FXI levels and severity of bleeding. We and others have reported a decreased fibrinolytic resistance in FXI-Def, allegedly attributable to a reduction of thrombin-dependent antifibrinolytic activities.

Aims: We investigated the mechanism behind increased lysability of FXI-Def clots.

Methods: Eighteen patients with FXI-Def (1–58%) and 17 matched controls were studied. Fibrinolytic resistance was evaluated by the lysis time of plasma clots exposed to 30 ng mL⁻¹ t-PA, thrombin generation by CAT and TAFI activation by two-stage fibrinolytic assay.

Results: When clotting was induced by 0.5 pM tissue factor (TF), FXI-Def samples displayed less thrombin and TAFIa generation and shorter lysis time than controls ($P < 0.01$ for all). The contribution of TAFI to fibrinolysis (lysis time difference upon PTCI addition) was smaller in FXI-Def patients ($P = 0.01$). A 100-fold higher concentration of TF (to bypass FXI) abolished the difference in thrombin generation but not the difference in lysis time between FXI-Def and controls ($P = 0.05$). Normalization of FXI level by a FXI concentrate increased thrombin generation ($P = 0.04$) but not the lysis time of FXI-Def plasma (60.3 ± 10.0 vs 61.2 ± 9.7). To make fibrinolysis independent of patient's coagulation, clots were induced by purified thrombin and a high concentration of FXa inhibitor. Surprisingly, also under this condition the lysis time was shorter ($P = 0.017$) and less TAFIa was generated in FXI-Def plasma than in controls

($P = 0.0002$). Finally, upon addition of TAFIa (2 nM), the lysis time of FXI-Def plasma (clotted with reptilase) prolonged significantly less than control plasma (80 ± 59 vs 187 ± 58 min, $n = 5$, $P = 0.02$).

Conclusion: An impaired TAFI pathway, largely unrelated to clotting deficiency, appears the main cause of decreased fibrinolytic resistance in FXI-Def. The mechanism interfering with TAFI activation/activity remains to be identified.

Disclosure of Interest: None declared.

PO504-MON

Impact of assay method on clinical dosing of a purified factor X concentrate

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Background: Factor X functional activity is measured by either a factor-specific prothrombin time assay (clotting assay), or a chromogenic assay measuring chromophore release from a peptide substrate. Although the Ph.Eur. compendial chromogenic assay is used to label factor concentrates in Europe, clotting assays are mainly used to diagnose and treat factor X deficiency.

Aims: To compare factor X clotting assay and chromogenic assay for optimised dosing of a high purity factor X concentrate (FACTOR X).

Methods: Factor X activity by clotting assay and chromogenic assay were compared using statistical methods (Bland and Altman, 1999). Statistical bias (tendency of one assay to exceed the other) was calculated for FACTOR X and for plasma samples from a clinical trial in hereditary factor X deficient patients.

Results: All datasets showed a bias between the chromogenic and clotting assays. The (chromogenic/clotting) ratio was 0.90–1.12. Generally the bias was lower (0.90–1.02) when the two assays used the same standard preparation, rather than using the different plasma standards supplied with each assay kit (1.05–1.12). Assay design influenced results. Interpolation from a 6-point standard curve showed a bias (ratio 1.06) compared to analysis of the same data by parallel line analysis with a 3-point standard line.

Conclusion: Bias between the chromogenic and clotting assays varied depending on the standard preparation and analytical method used. The bias between assays was also within validated accuracy and precision. The small bias between the assays is unlikely to be clinically significant even when BPL FACTOR X potency is assigned by chromogenic assay and patient factor X levels are measured by clotting assay. Recalculation of the clinical trial incremental recovery (2.07 IU dL⁻¹ per IU kg⁻¹ using only clotting assay) using a concentrate potency assigned by chromogenic assay and patient plasma levels measured by clotting assay caused only a small adjustment to 2.00 IU dL⁻¹ per IU kg⁻¹.

Disclosure of Interest: None declared.

Regulation of gene expression in vascular cells

PO506-MON

Biogenesis, subcellular localization and mobilization of two abundant platelet thiol isomerases

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Background: Thiol isomerases facilitate the rearrangement of protein disulphide bonds that are required for the correct folding of secreted proteins. While typically localized in the endoplasmic reticulum (ER), in platelets they are believed to act at the cell surface to regulate platelet activation, although their origins and distribution in these cells has caused contention.

Aims: This study aimed to explore the fates of PDI and ERp57 from their synthesis in megakaryocytes to subcellular location in platelets, and the mechanism of release during platelet activation.

Methods: Using confocal microscopy, we explored the biogenesis PDI and ERp57, and followed their fate from immature megakaryocytes (MK) to mature platelets, and their release to the platelet surface.

Results: Throughout MK development, PDI and ERp57 were organized into punctuate structures in the cytoplasm which become progressively smaller with cell maturation and were trafficked to proplatelets. PDI and ERp57 were transported separately from α -granule cargo proteins and from calnexin, an ER chaperone. In mature human or mouse platelets they were not found in α -granules, but were co-distributed in distinct granular compartments that were present in Nbeal2^{-/-} platelets that lack α -granules. PDI, which was also absent from lysosomes and δ -granules, has been reported to localize with TLR9 in electron-dense regions termed T-granules. We did not observe a significant degree of co-distribution between PDI and TLR9, suggesting that this thiol isomerase largely resides in a compartment distinct from T-granules. When platelets were activated, thiol isomerases were secreted to the platelet surface and this, similar to α -granule secretion, was regulated by the polymerization of actin.

Conclusion: We conclude that PDI and ERp57 are synthesized early in megakaryocyte development and packaged into secretory compartments that are distinct from known granules and from where they are released to the platelet surface via an actin-dependent mechanism.

Disclosure of Interest: None declared.

Reproductive issues I

PO507-MON

Risk factors for obstetric bleeding in von willebrand disease (VWD)

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Background: Women with VWD face bleeding risks during pregnancy, delivery, and the postpartum period. Post partum hemorrhage, for example, occurs in 10–21% of controls compared to 31–59% of

women with VWD. Few cohorts exist, however, to assess obstetrical risk factors in this population. The Centers for Disease Control and Prevention's Universal Data Collection Surveillance System Female Module offers an opportunity to quantify predictors of obstetric bleeding.

Aims: To build a multivariable regression model to identify characteristics of women with VWD who are at higher risk for pregnancy-related bleeding.

Methods: Gynecologic and reproductive history and outcomes data for 174 women diagnosed with VWD who reported at least one pregnancy were extracted from all 30 participating US HTC's from 2009–2011. We examined 18 independent variables as possible predictors of bleeding with miscarriage, antepartum bleeding, and/or postpartum bleeding. Independent variables with $P < 0.15$ were included in the multivariable model.

Results: One-hundred and seven (61%) of 174 women reported pregnancy-related bleeding. Of the 62 who reported miscarriage, 52 (84%) had bleeding. Rates of antepartum and postpartum bleeding were 31% and 48% respectively. Univariate analysis revealed 8 variables associated with bleeding: age at diagnosis, history of anemia, cumulative number of other gynecologic abnormalities, cumulative bleeding symptom score, cumulative provider intervention for bleeding score, and medication/device use, surgery, and blood/factor product use for bleeding or menorrhagia. After removing highly correlated variables, 3 were incorporated into the exploratory multivariable model. Using the minimum Bayesian information criterion, the final model included history of anemia (OR 4.94, 95% CI 1.30–21.63, $P = 0.019$) and bleeding symptom score (OR 1.36, 95% CI 1.14–1.66, $P < 0.001$).

Conclusion: These data suggest that history of anemia and bleeding symptom score, if prospectively validated, may identify women at risk for VWD-associated obstetric bleeding.

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PO508-MON

Placental insufficiency resolution with full anticoagulation in patients with prior bad obstetric outcome and dyslipidaemia during pregnancy

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Background: Dyslipidaemia may be a cause of bad pregnancy outcome.

Aims: To describe a successful treatment of a patient with placental insufficiency and dyslipidaemia with anticoagulation using enoxaparin.

Methods: Case Report.

Results: Case report: B.E.F., 27 years old, white, two pregnancies, one vaginal delivery in december 2013 after intrauterine fetal death due to intra uterine growth restriction and placental insufficiency at 28 weeks. (Placental histology with multiple areas of infarction, 2% gross).

The postpartum research revealed an increased lipoprotein A (20), negative tests for thrombophilia and total cholesterol and triglyceride levels in the normal range (159 e 95 mg dL⁻¹ respectively)

Pregnancy began making use of enoxaparin 40 mg day⁻¹, ASA 100 mg day⁻¹, folic acid 5 mg day⁻¹, vitamin D, calcium 500 mg day⁻¹. In the twenty-ninth week of pregnancy, the percentile of fetal weight decreased (percentile was 32 at 25 w and 10 at 29 w) and increased umbilical artery resistance. It was decided to increase enoxaparin to 60 mg day⁻¹. In the thirtieth week a new and significant

increase in the umbilical artery resistance occurred (A/B 5.1) and cholesterol levels and triglyceride ($283 \pm 448 \text{ mg dL}^{-1}$ respectively) increased too. A decision to full anticoagulation was taken (enoxaparin to 120 mg day^{-1}). In the next two weeks the fetal growth was recovered and uterine artery resistance decreased.

A healthy newborn was delivered at 37 w with appropriate weight for gestational age (2460 g), with good performance in the nursery. The patient remained with enoxaparin 60 mg day^{-1} in the first two weeks postpartum.

Conclusion: Dyslipidaemia may be a cause of recurrent fetal loss and placental insufficiency. If prophylactic and early anticoagulation do not prevent placental insufficiency, therapeutic anticoagulation may resolve this problem as described in this case report.

Disclosure of Interest: None declared.

PO509-MON

D-dimer measurement has no diagnostic value during pregnancy – a longitudinal study of 4314 samples from 791 healthy women

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Background: Several reports have shown that the D-dimer concentration increases progressively throughout normal pregnancy, hampering the usefulness of the conventional cut-off point above 0.5 mg L^{-1} (FEU) for dismissing thromboembolism. In spite of this fact, some still recommend change in D-dimer values as an aid in diagnosing thromboembolisms in pregnant women.

Aims: To investigate if individual changes in D-dimer values during pregnancy are small enough to be helpful in evaluating thromboembolisms in pregnant women, provided an initial value is obtained early in pregnancy.

Methods: A total of 791 women, healthy and expected to experience normal singleton pregnancies, were recruited among 2147 women attending first trimester screening. D-dimer values were measured on the STA-R Evolution coagulation analyzer with STA Liatest D-DI #00515 reagent (Diagnostica Stago, Asnieres Sur Seine, France) repeatedly during the pregnancy, at active labor, and one and two days postpartum. The obtained D-dimer values were normalized through transformation into percentiles for the relevant gestation age week. The range in percentile points was evaluated for each individual woman.

Results: Plasma D-dimer values during pregnancy deviated about 20 percentile points in about half the women, and in one out ten women the D-dimer values deviated more than 50 percentile points.

Conclusion: Due to the high degree of fluctuations in D-dimer percentile points within each individual women during normal pregnancy, longitudinal D-dimer measurements are of no clinical use in evaluation thromboembolic events during pregnancy, nor are solitary D-dimer measurements.

Disclosure of Interest: None declared.

PO510-MON

Men with factor V Leiden have increased sperm counts

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Background: Factor V Leiden (FVL) is common amongst Caucasians, despite its association with venous thromboembolism and miscarriage. It is therefore presumed to also convey an evolutionary benefit. Male carriers have increased fecundity and we previously observed a statistically non-significant increase in total sperm count in FVL carriers. For the prothrombin G20210A mutation (PGM), the second most prevalent inherited thrombophilia, such an association has not been investigated.

Aims: To assess if FVL and/or PGM are associated with increased total sperm count.

Methods: We report on a consecutive cohort of 1473 men of couples presenting with subfertility to our academic hospital in the Netherlands, of which 908 men were previously reported on. Men with azoospermia or a known cause for oligozoospermia were excluded. Genotyping was done with PCR. Differences in semen parameters were tested with Kruskal-Wallis test.

Results: Here, we present preliminary data, as inclusion is ongoing. The mean age was 37.2 years. The prevalence was 4.2% for FVL and 1.9% for PGM. Median total counts were 235×10^6 (IQR 89 – 330) for FVL carriers vs. 159×10^6 (IQR 57 – 297) for non carriers (*P* value 0.060). Sperm parameters did not differ between PGM carriers and non carriers.

Conclusion: Our preliminary results again suggest an increased sperm count in men with FVL. We will report complete data on the conference. Increased sperm count might very well explain the high prevalence of FVL in the general population despite its detrimental health effects of venous thromboembolism and miscarriage. The basis for the association remains to be elucidated. If coagulation related, it might be mediated by an effect on protein C inhibitor, which has been implicated in spermatogenesis and fertility. However PGM, which has similar effects on coagulation, was not associated with sperm count. A plausible alternative explanation is genetic linkage of FVL with a nearby locus that affects spermatogenesis.

Disclosure of Interest: None declared.

PO511-MON

Incidence of venous thrombosis in pregnancies after spontaneous and art conception – an Italian cohort study

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Background: Assisted Reproductive Technologies (ART) may increase thrombotic risk after successful cycles. Ovarian Hyper Stimulation Syndrome (OHSS) or thrombophilias could increase this risk.

Aims: To determine 1) the incidence of venous thrombosis in successful ART cycles and compared it to that of general population conceiving spontaneously and 2) the weight of potential risk factors.

Methods: A cohort of 998 women approaching ART between April 2002 and July 2011 consecutively referred by local Fertility Clinics was compared to a cohort of women who consecutively delivered ($n = 3339$) at our Hospital after spontaneous conception (2010–2012). Among women undergone ART, 234 achieved 1 clinical pregnancy. For women who conceived spontaneously, information on the diagnosis of venous thrombosis was obtained by linkage to our Institutional patient register by using specific codes according to the International Classification of Diseases, 9th revision (ICD-9).

Results: Overall, 3 venous thromboses in pregnancies after ART and 11 in the spontaneous ones were recorded (Two-tailed Fisher exact test: $P = 0.06$, OR = 3.9, 95%CI = 0.87–15.3). After the exclusion of superficial thromboses, the incidence of venous thromboembolism was 2/234 in pregnancies from ART (8.5 ‰) and 6/3339 (1.8 ‰) in the spontaneous ones ($P: 0.09$). Among women carrying thrombophilias ($n = 24$) only 1 showed a venous thrombosis. An antithrombotic prophylaxis was prescribed in 23/234 (9.8%) women undergone ART. The incidence of thrombotic events with/without prophylaxis with LMWH was not statistically significant (thromboses: 1/23 vs 2/211, $P: ns$). Among 234 successful cycles, 10 (4.3%) were complicated by OHSS. LMWH was prescribed because of OHSS in 3 out of 23 (13%) treated cases; furthermore, an OHSS was recorded in 7 out of 211 (3.3%) untreated pregnancies.

Conclusion: Women undergone ART cycles have a slightly higher risk of venous thrombosis as compared to pregnant women who conceive spontaneously.

Disclosure of Interest: None declared.

PO512-MON

Post-partum prophylaxis in practice

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Background: Venous thromboembolism (VTE) is one of the leading causes of maternal mortality in developed world and is highly preventable. Population studies identified risk factors for VTE during post-partum (PP) and guidelines for thromboprophylaxis are available in Western countries. Nevertheless, recommendations are of low level of evidence with several differences in care.

Aims: Assessment of adherence to recommendations in thromboprophylaxis prescription during post-partum and analysis of prescription errors.

Methods: We compared thromboprophylaxis practices to recommendations, in consecutive women who delivered in Brest from April 2013 to March 2014. General characteristics, VTE history and pregnancy outcomes were recorded. Women were classified at low, intermediate and high risk for VTE during PP. Thromboprophylaxis prescription was analysed for women classified at intermediate or high risk, all women with caesarean section (CS) and a sample of 90 women at low risk without CS.

Results: Thousand-eight-hundred and eleven women were evaluated, 393 were over 35 years and 254 were obese. 298 women were classified at intermediate or high risk for VTE during PP (267 due to CS and additional risk factors, 31 for other medical reason). 140 women had CS without additional risk factor. Prescription was adequate for 33% of women at risk, 6.9% of women requiring extended prophylaxis and 15 of 16 women with past history of VTE. 97% of women with low risk CS were overtreated. None of the 90 sampled low risk women without CS received prophylaxis. Association of CS and age over 35 (36% of women) identified 98% of women with indication for extended prophylaxis.

Conclusion: Adherence to recommendations was low; patients combining low risk factors were undertreated. Complexity of guidelines and scientific weakness of data are barriers to adherence of practitioners. Simplified risk scores and clinical studies are needed to identify women at risk and confirm the best preventive strategy.

Disclosure of Interest: None declared.

PO513-MON

The M2 haplotype within the annexin A5 gene and antiphospholipid antibodies in unselected pregnant women- a prospective hospital- based study

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Background: Antiphospholipid antibodies (aPL) and the M2 haplotype in the annexin A5 (ANXA5) gene are recognized causes of obstetric complications. It is known that aPL are directed against annexin, that is particularly abundant in pregnancy. However, it is not known whether carriers of the M2 haplotype are more likely prone to the development of aPL.

Aims: In a general obstetric population 1) to determine a possible relationship between the carriership of the M2 haplotype and the presence of aPL 2) to test the association between the M2 haplotype with/without aPL and pregnancy loss.

Methods: From an initial cohort of 5345 DNA and serum samples of pregnant women consecutively admitted to 14 hospitals of the Campania region (Italy), between 1st November 2000 and 31st January 2002, 608 samples were available to investigate the presence of IgG/IgM anticardiolipin (aCL), IgG/IgM anti human β 2-Glycoprotein I (a β 2GPI) antibodies and the M2 haplotype in the ANXA5 gene. Cut-off values for medium/high titers were 20 GPL/MPL for both IgG/IgM aCL and a β 2GPI. Obstetric outcomes of the ongoing pregnancy were collected.

Results: Overall, 267/608 (43.9%) pregnant women tested positive for aPL and/or M2 haplotype: 126/267 (47.2%) showed the presence of medium/high titers aPL, 117/267 (43.8%) carried the M2 haplotype, 24/267 (9.0%) showed both. Among the carriers of the M2 haplotype ($n = 141$), 24 (17%) tested positive for aPL, as compared to 25.5% (117/459) women without aPL carrying the M2 haplotype ($P: ns$). Thirty-six out of 608 (5.9%) pregnancies resulted in pregnancy loss; in 16 (44.4%) of them the M2 haplotype or aPL were detected. Among women who delivered an at term neonate ($n = 572$), 251 (43.9%) carried the M2 haplotype and/or aPL.

Conclusion: In general obstetric population the carriership of the M2 haplotype was not associated with medium/high titers aPL. No association was shown between the carriership of M2 and/or aPL and pregnancy loss. Therefore, in at low-risk pregnant women these tests are not recommended.

Disclosure of Interest: None declared.

PO514-MON

Age of menarche and risk of venous thromboembolism

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Background: Menarche is the central event in the onset of female puberty and a sign of ovarian activity. It is the moment which initiates exposure to endogenous estrogen production. The time of menarche depends on genetic and environmental factors, and has been reported to affect cardiovascular risk. To date, little is known about age of menarche and risk of venous thromboembolism (VTE).

Aims: To assess whether age of menarche is associated with risk of VTE.

Methods: All female participants from the MEGA case-control study, a study into risk factors for VTE ($n = 2679$ cases, 3383 controls), were included. Age of menarche was recorded as reported by the participants on a questionnaire and categorized for the following ages: ≤ 10 , 11, 12, 13, 14, 15, 16 and ≥ 17 . Menarche at the age of 14 was chosen as reference category. Logistic regression models were constructed to estimate odds ratios (ORs) with 95% confidence intervals (CIs).

Results: Age of menarche was available for 2427 VTE cases and 3149 controls. The ORs for the early menarche categories ≤ 10 , 11 and 12 years were 1.1 (95%CI, 0.8–1.6), 1.3 (95%CI, 1.1–1.6) and 1.2 (95%CI, 1.0–1.4), respectively. For menarche at age of 13 the OR was 1.0 (95%CI, 0.9–1.2). For later menarche in the categories 15, 16 and ≥ 17 years the corresponding ORs were, 1.1 (95%CI, 0.9–1.3), 1.1 (95%CI 0.8–1.4) and 1.3 (95%CI, 0.9–1.8), respectively.

Conclusion: Age of menarche is associated with VTE risk in later life, possibly in a U-shaped relationship. Possible explanations are endogenous estrogen exposure related to age at menarche or confounding factors such as socio-economic class.

Disclosure of Interest: None declared.

PO515-MON

Effect of prophylaxis with lmwh on implantation in women undergoing assisted reproductive procedures (IVF or ICSI): a prospective randomized study

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Background: An experimental strategy employed to improve embryo implantation and possibly positive pregnancy outcome was attributed to the use of heparins, particularly low molecular weight heparin (LMWH).

Aims: The aim of the study is to test whether prophylactic dose of LMWH could improve the implantation rate in women with inherited thrombophilia or a history of two or more than two consecutive implantation failures.

Methods: Inclusion criteria are: heterozygosity of inherited common thrombophilias (FVL, PTm), hetero/homozygosity of MTHFR, two or more than two previous implantation failures, age > 18 , informed consent. Exclusion criteria are: deficiency of antitrombin, protein C, protein S, antiphospholipid antibodies, homozygosity or double heterozygosity for FVL/PTm, history of venous thromboembolism. Women enrolled in the study are randomly divided into two groups. One group receives prophylaxis with LMWH (dalteparin 5000 UI die⁻¹) (cases), the other does not (controls).

Results: From September 2012, 81 women gave their consent to participate in the study and have completed a course of procedure: thirty-three in the group of cases (mean age 39 years, four homozygous for MTHFR C677T, seventeen heterozygous for MTHFR C677T, three heterozygous for FVL, two double-heterozygous for FVL/MTHFR, seven with more than two implant failure) and forty-eight in the control group (mean age 40 years, five homozygous for MTHFR C677T, twenty-eight heterozygous for MTHFR C677T, two heterozygous for PTm, one double-heterozygous for FVL/MTHFR, twelve with more than two implant failure). Thirteen implants occurred in the group of cases and six occurred in the control group. The odds ratio was 3.15 (95% CI 1.33 to 7.45) [RRR 0.32 (95%CI 0.13 to 0.75)].

Conclusion: The results of this study shows a potential advantage of anticoagulant prophylaxis in the improvement of the implantation rate in MAP.

Disclosure of Interest: None declared.

PO516-MON

Prediction of venous thromboembolic related fetal losses by a new genetic risk score

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Background: Inherited thrombophilia is the leading cause of maternal venous thromboembolism (VTE) and is associated with an increased risk of fetal loss. Nowadays, Factor V Leiden (FVL) and Prothrombin G20210A (PT) are the genetic variants tested to assess fetal loss risk associated to venous thromboembolism (VTE), but with a very low sensitivity. Thrombo inCode (TiC) is a new VTE predicting tool that combines genetic and clinical information.

Aims: In the present study, we have analyzed whether TiC provides a prediction of fetal loss associated with VTE with a better accuracy than the method nowadays in use (FVL+PT).

Methods: Retrospective case-control study that included 72 cases with at least two idiopathic fetal loss and 36 controls with at least one pregnancy with success delivery. All participants provided written informed consent and the study was approved by the ethics committee. Two weight scores were used in all subjects, one with FVL+PT and another with TiC (including genetic variants FVL, PT, F5 rs118203906 and rs118203905, F12 rs1801020, F13 rs5985, SERPINC1 rs121909548, SERPINE10 rs2232698 and A1 blood group rs8176719, rs7853989, rs8176743, rs8176750) plus VTE risk associated to pregnancy. Prediction capacity was assessed in terms of discrimination, measured as area under the receiver operating characteristic curve (AUC), and sensibility and specificity.

Results: TiC showed better discrimination (AUC) compared to FVL+PT (AUC: TiC 0.65, CI 0.55–0.74 vs 0.55, CI 0.45–0.64, $P = 0.03$). TiC had a better sensitivity (50, CI 38.0–62.0 vs 9.72, CI 4.0–19.0, $P = 0.01$ TiC and FVL+PT, respectively). Specificity was similar for TiC than for FVL+PT (100, CI 90.3–100 vs 94.4, CI 81.3–99.3, FVL+PT and TiC, respectively). In the 27 fetal necropsy obtained there was no correlation between necropsy findings and TiC score.

Conclusion: TiC predicts fetal losses associated to VTE significantly better than FVL+PT and can have a clinical use in preventing fetal losses.

Disclosure of Interest: E. Salas Employee of: Gendiag.exe, J. Paramo: None Declared, R. Lecumberri: None Declared, A. Alfonso: None Declared, K. Guillen Employee of: Gendiag.exe, S. Pich Employee of:

Gendiag.exe, O. Casagran Employee of: Gendiag.exe, A. Ferro Employee of: Ferrer inCode, J. Soria: None Declared.

PO517-MON

Monitoring of coagulation activation marker and application of LMWH in pregnancy

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Background: The application of low molecular weight heparin (LMWH) in pregnant women with thrombophilia or thrombosis in anamnesis is controversial. No guidelines exist about when to start with LMWH. We observed pregnant women using D-dimer, fibrinogen (FM), and prothrombin fragment f1 + 2 levels and thrombin generation (TG). In cases of a significant increase or a value higher than expected LMWH was started.

Aims: The study aimed to find a time point for LMWH therapy in pregnant women with thrombophilia.

Methods: Two-hundred and eight pregnant women w/o LMWH were investigated, 56% of them with thrombophilia and 44% with thrombosis in anamnesis. The calculations were done based on mean levels of D-dimer and f1 + 2 (Siemens Healthcare Diagnostics), FM and TG (Diagnostica Stago) corresponding to the duration of pregnancy.

Results: All measured parameters were elevated in pregnancy, but there are no significant differences between women with thrombophilia and such with thrombosis in anamnesis. The increase of D dimer is $0.04 \mu\text{g mL}^{-1} \text{ week}^{-1}$ and of f1 + 2 is $0.11 \text{ pM week}^{-1}$. D-dimer significantly decreased under LMWH therapy. In average patients with thrombosis received LMWH from week 15 whereas patients with thrombophilia started in week 20. 46% received LMWH in post partum period only. TG is significantly lower in the LMWH group and shows nearly the same kinetics like D-dimer. F1 + 2 levels increase non-significantly under LMWH. Platelet counts between patients w/o LMWH are comparable.

Conclusion: The application of LMWH decreases the level of coagulation activation in pregnancy. The decrease in D-dimer and TG confirms the decision for application of LMWH in cases without any guidelines and thrombophilic disposition in pregnancy. Based on median increases per week for each activation marker this procedure could be a method recognizing the women at extra high risk in pregnancy. Under this procedure no clinical events were observed.

Disclosure of Interest: None declared.

PO518-MON

Mechanism of the increased thrombosis risk induced by blood loss during caesarian section

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Background: Pregnancy-associated venous thromboembolism (VT) is the leading cause of maternal mortality in developed countries. The risk is higher postpartum, after caesarian section (CS) and further increases after delivery with high blood loss.

Aims: To investigate the mechanisms leading to an increased risk of VT after surgical delivery with high blood loss. As during pregnancy almost all coagulation factors increase and anticoagulant proteins decrease, blood loss will likely result in the normalization of coagulation factors, whereas the deficiency in anticoagulant proteins (e.g. pro-

tein S, TFPI) will become more pronounced, leading to an increased risk on VT.

Methods: Fifty pregnant women undergoing a primary CS will be enrolled in the study. Blood samples will be collected before CS, immediately after, 1, 6, 24 h and 5 days after surgery. Blood loss and infusions during CS will be exactly evaluated. The study is approved by Ethical Committee of University of Magdeburg, Germany and began in August 2014. All participants give written informed consent. At the moment of the abstract submission 35 participants are enrolled in the study and the enrollment will be completed by March 2015.

Results: Thereafter thrombin generation will be measured in plasma at different experimental conditions to assess function of different coagulation and anticoagulant pathways. Major determinants of thrombin generation will be identified. Blood loss during CS will be correlated with the changes in thrombin generation parameters and coagulation factors and anticoagulant proteins. Further, the results will be fortified by *in vitro* experiments, simulating the changes in coagulation induced by blood loss.

Conclusion: The study will reveal the mechanisms of hypercoagulability induced by blood loss during CS. Additionally, optimization of the infusion therapy will be suggested, taken into account that infusion may increase haemodilution and shift the balance between coagulation and anticoagulants even further.

Disclosure of Interest: None declared.

Risk factors venous thrombosis I

PO519-MON

Identifying common and rare genetic variants associated with serum levels of atherosclerotic biomarker proteins

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Background: Genetic variants can be used to identify causal relationships between biomarkers and diseases, such as between atherosclerotic biomarkers and venous or arterial thrombosis. To identify new common and rare genetic variants associated with protein biomarkers, we are identifying loci genotyped through whole exome sequencing that are associated with 50 atherosclerotic biomarkers.

Aims: To identify common (minor allele frequency >0.05) genetic variants and genes that carry multiple rare variants that are associated with serum levels of atherosclerotic biomarkers.

Methods: We have performed exome sequencing or array genotyping on DNA from 330 individuals recruited from the general population in Tromsø, Norway (the Tromsø Study). Serum from these individuals was isolated at study entry and profiled for the levels of 50 proteins involved in platelet activation, degradation of the extracellular matrix, and immune signaling. For each protein, we have identified variation in or near its respective gene to test for association. We used a mixed model to test for associations with common variants and a sequence-kernel association test to identify genes that carry multiple rare variants associated with protein levels. Using significant sites, we will explore the downstream consequences of these associations by testing how they are associated with the other 49 proteins to identify potential

pathway relationships. This study was approved by the research ethics committee and all subjects gave informed written consent.

Results: We have identified seven known and one novel association between common genetic variants and their protein levels. We are currently performing rare variant analysis before analyzing variants for pathway associations.

Conclusion: We have identified common genetic variation associated with the serum levels of 8 atherosclerosis biomarkers. In the future, we will use these variants to investigate the molecular mechanisms underlying cardiovascular diseases, including venous and arterial thrombosis.

Disclosure of Interest: None declared.

PO520-MON

Comparison of thrombin generation test and markers of coagulation activation in uncomplicated pregnancy and pregnancy-related venous thromboembolism

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Background: Pregnancy, a well-established risk factor for venous thromboembolism (VTE), is associated with a state of hypercoagulability. During pregnancy, it is important to investigate the role of haemostatic risk factors of VTE disease with sensitive and specific biological markers of thrombosis. Thrombin generation (TG) test is a global dynamic assay, which automatically investigates all stages of thrombin generation and inactivation. D-dimer, prothrombin fragments 1 + 2 (F1 + 2), thrombin-antithrombin complexes (TAT) and fibrin monomer complexes (FMC) are defined as markers of coagulation activation.

Aims: Firstly, this study aimed to compare TG test in pregnancy-related VTE and uncomplicated pregnancy. Secondly, the evolution of fibrinogen and markers of coagulation activation was studied in both groups and compared with TG test evolution. The final goal of this study was to assess TG assay in pregnancy-related VTE.

Methods: In this study, 106 consecutive uncomplicated pregnant women and 134 pregnancy-related VTE, followed retrospectively and longitudinally, were enrolled. TG test was performed using platelet-poor plasma, final concentrations of 5pM tissue factor and 4 µM phospholipids. Fibrinogen and markers of coagulation activation were assessed for each patient.

Results: During uncomplicated pregnancy and untreated pregnancy-related VTE, TG was early increased, and stabilized during the second month. Anticoagulant treatment disturbed TG test. Markers of coagulation activation, except FMC, increased significantly throughout normal pregnancy and untreated pregnancy-related VTE. FMC varied during pregnancy with wide inter-individual variations. No correlation was demonstrated between TG parameters and other activation markers.

Conclusion: The utility of TG test is questioned, in our experimental conditions, to detect a pathological hypercoagulability state during pregnancy. To assess the follow-up of pregnancy-related VTE, we recommend to perform D-dimer, F1 + 2, clinical examination and medical imaging when needed.

Disclosure of Interest: None Declared.

PO521-MON

All cause mortality and use of antithrombotics within 90 days of discharge in acutely ill medical patients

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Background: Conflicting evidence exists regarding predictors of mortality and the benefit of antithrombotic therapy on mortality in hospitalized acutely-ill medical patients.

Aims: To identify characteristics associated with receiving antithrombotic therapy after discharge and compare the risk of all-cause mortality within 90 days post-discharge among patients who did and did not receive antithrombotics.

Methods: We conducted a large ($n = 327,578$) retrospective claims analysis of patients aged ≥ 40 years hospitalized ≥ 2 days for nonsurgical reasons between 2005 and 2009 using the US based HealthCore Integrated Research Database. Antithrombotic use post-discharge was captured from pharmacy claims. All-cause mortality was determined using the Social Security Death Index and cause of death was identified from the National Death Index database. Kaplan-Meier survival curves were generated and Hazard ratios (HR) for mortality risk were estimated using multivariable Cox proportional hazards models.

Results: Patients prescribed either anticoagulants or antiplatelets post-discharge had a lower risk of all-cause mortality. For anticoagulants, the most significant predictors of mortality were neoplasms (HR 1.606, 95% Confidence Interval [CI] 1.528–1.688), liver disease (HR 1.582, 95% CI 1.462–1.713), anticoagulant omission (HR 1.577, 95% CI 1.418–1.754), gastrointestinal (GI) or respiratory tract intubations (HR 1.507, 95% CI 1.318–1.724), and blood dyscrasias (HR 1.444, 95% CI 1.381–1.510). For antiplatelets, the most significant predictors of mortality were antiplatelet omission (HR 3.659, 95% CI 3.266–4.099), liver disease (HR 1.558, 95% CI 1.440–1.687), neoplasms (HR 1.555, 95% CI 1.479–1.635), GI or respiratory tract intubations (HR 1.481, 95% CI 1.295–1.694), and blood dyscrasias (HR 1.426, 95% CI 1.364–1.491).

Conclusion: The described risk factors for all-cause mortality may guide future studies assessing potential mortality benefits with antithrombotics in specific subsets of medical patients.

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PO522-MON

Risk of venous thrombosis and coagulation profile in Dutch natives and immigrants in the Netherlands: results from the mega studyRezende S¹, Lijfering W², Bos M², Meijers J³, Rosendaal F² and Cannegieter S²¹Universidade Federal de Minas Gerais, Belo Horizonte, Brazil;²LUMC, Leiden; ³University of Amsterdam, Amsterdam, Netherlands

Background: Venous thrombosis (VT) rates seem to vary between individuals with different ethnic backgrounds. Several studies suggest a lower incidence of VT in Asian than in Caucasian populations. However, most of these studies have used administrative databases sources and no study has yet investigated the mechanism behind this variation in risk.

Aims: To investigate the risk of VT in several first and second generation immigrant groups in the Netherlands and to study the mechanism for differences in risk.

Methods: The study was performed in a large population-based case-control study (MEGA study) and included individuals aged 18–70 originating from 76 countries. Participants completed detailed questionnaires on putative risk factors for VT and lifestyle-related factors. Coagulation profile was assessed by measurement of anticoagulant, procoagulant, and fibrinolytic proteins.

Results: Dutch and immigrant individuals ($n = 3285$ and $n = 475$ cases, respectively, and $n = 2273$ and $n = 326$, controls, respectively) were analyzed. The risk of VT varied in different populations. Compared with native Dutch, Eastern Europeans showed the highest (odds ratio [OR], 2.2; 95% CI, 1.2–4.1) and Asian immigrants the lowest risk (OR, 0.6; 95%CI, 0.5–0.8), which was most pronounced in East/Southeast Asian immigrants (OR, 0.4; 95% CI, 0.3–0.6). Levels of hemostatic proteins in native Dutch and East/Southeast Asian controls showed differences for thrombin-activatable fibrinolysis inhibitor (TAFI) activity levels which were lower in the latter (adjusted mean difference, –10%; 95%CI, –16% to –3%). The protective effect in East/Southeast Asian immigrants disappeared after adjustment for TAFI levels (OR, 1.1; 95% CI, 0.6–1.9).

Conclusion: Thrombotic risk varies within different groups of immigrants and is lowest in East/Southeast Asians, in whom low levels of TAFI were found. We postulate that the reduction in TAFI levels explains the decreased risk of VT in East/Southeast Asians which mechanism requires further investigation.

Disclosure of Interest: None declared.

PO523-MON

Impact of tornadoes on hospital admissions for venous thromboembolism and cardiovascular eventsCasanegra AI¹, Silva F¹, Shapiro A², Phan M³, Hawkins B¹, Li J⁴, Stoner J⁴ and Tafur AJ¹¹Cardiovascular Section, Department of Medicine; ²School of Meteorology; ³Hematology and Oncology Section, Department of Medicine; ⁴Department of Biostatistics and Epidemiology, College of Public Health, University Of Oklahoma, Oklahoma City, USA

Background: Natural disaster can impact human health. There is a paucity of data describing venous thromboembolism (VTE) and cardiovascular events (CVEs) after tornado outbreaks.

Aims: To study the effects of tornadoes on the incidence of VTE and CVE at a tertiary care institution.

Methods: ICD-9 search of hospital admission records from a single center in a tornado-prone area three months before and after a severe 2013 tornado outbreak (two high intensity tornadoes within a week)

were abstracted. To control for seasonal variation, we also abstracted data from the same period of the prior year (control). Hospital admissions for VTE and CVE (including acute myocardial infarction, stroke and VTE) were summated by zip codes, and compared by time period. **Results:** There were 22,607 admissions analyzed, of which 6705 (30%) and 7980 (35%) were during the pre- and post-tornado time frames respectively. There were 100 VTE in the control, 91 in the pre-tornado and 118 in the post tornado periods. Combined CVE were 344, 317 and 364 (controls, pre tornado and post tornado periods respectively). There was no difference in the odds of having a VTE during the post-tornado season compared with the control (OR=0.85, 95% CI: 0.65 to 1.11, $P = 0.24$) or the pre tornado (OR=0.92, 95% CI: 0.70 to 1.21, $P = 0.54$). Similar results were found for the combined CVE comparing the post tornado with control (OR=1.05 95% CI: 0.91 to 1.22, $P = 0.50$) or with pre-tornado (OR=0.96, 95% CI: 0.83 to 1.12, $P = 0.63$). The prevalence of CVE subtypes was not different among the 3 time periods: 1.3%>1.5% for VTE, 1%>1.2% for CAD, and 2%>2.2% for stroke. There were differences in CVE by zip codes, but they were constant along the three time periods.

Conclusion: The tornado outbreak did not increase the prevalence of VTE or CVE. In contrast to the effect of hurricanes, implementation of a healthcare policy change directed toward the early treatment and prevention of CVE after tornadoes does not seem warranted.

Disclosure of Interest: None declared.

PO524-MON

Joint effects of F5 gene variants and tall stature on VTE riskLønneberg TS^{1,2}, Smith E^{3,4}, Brækkan S^{1,2}, Horvei LD^{1,2}, Solomon T⁵, Wilsaard T⁶, Rosendaal F⁷, Frazer K^{3,4} and Hansen J-B^{1,2}¹K. G. Jebsen – Thrombosis Research and Expertise Center ^{TREC}, Department of Clinical Medicine, UiT – The Arctic University of Norway; ²Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway; ³University of California, San Diego, La Jolla; ⁴Department of Pediatrics, Rady's Children's Hospital, San Diego, USA; ⁵Biomedical Sciences Graduate Program, University of California; ⁶Department of Community Medicine, UiT – The Arctic University of Norway, Tromsø, Norway; ⁷Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands

Background: Factor V is an essential co-factor in the coagulation cascade, and FV gene variants (rs6025_T (FV-Leiden) and rs4524_T) are established inherited risk factors for VTE. Previous studies have shown that tall stature increases the VTE risk, probably due to gravity-induced stasis. We sought to investigate whether prothrombotic variants in the F5 gene had a joint effect with tall stature on VTE risk.

Aims: To investigate the joint effect of F5 gene variants (rs6025 and rs4524) and tall stature on VTE risk in a case-cohort recruited from a general population.

Methods: Cases with a first VTE ($n = 660$) and an age-weighted subcohort ($n = 1793$) sampled from the Tromsø 4 (1994–95) and 6 (2007–08) studies were included. Baseline DNA-samples were genotyped for F5 gene variants. Validated VTEs were registered up to Dec. 31st, 2012. The study population was stratified into tertiles (T) of body height with T1 < 164 cm and T3 ≥ 175 cm. Cox survival models were used to calculate hazard ratios (HR) for VTE across categories of body height and F5 gene variants adjusted for age, sex and body-mass index. Subjects in T1 without risk alleles served as reference. The regional committee for research ethics approved the study and all subjects gave informed written consent.

Results: Subjects with a tall stature (T3) had a 2.1-fold (95%CI: 1.6–2.9) higher VTE risk than short subjects (T1). Short subjects (T1) with

risk alleles for rs6025 or rs4524 had a 2.6-fold (95%CI; 1.7–4.0) and 2.2-fold (95%CI; 1.1–4.5) higher VTE risk, respectively, compared to those without risk alleles. Tall stature and risk allele for rs6025 or rs4524 was associated with a 3.4-fold (95%CI; 2.2–5.4) and 4.5-fold (95%CI; 2.1–9.5) higher VTE risk, respectively. A combination of risk alleles at the rs6025 and rs4524 sites and tall stature was associated with a 6.5-fold (95%CI; 2.9–14.8) higher VTE risk compared to short subjects without the risk alleles.

Conclusion: Our findings imply that risk alleles at FV gene sites increases the risk of VTE in tall stature.

Disclosure of Interest: None declared.

PO525-MON

Joint effect of obesity and risk alleles in the kng1 gene on risk of venous thromboembolism

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Background: Obesity is a well-established risk factor for venous thromboembolism (VTE). The single nucleotide polymorphism (SNP) rs710446_C in the kininogen 1 gene (KNG1) is associated with increased risk of VTE. There is, however, limited information about potential joint effects of obesity and risk alleles in the KNG1 gene on VTE risk.

Aims: To investigate the joint effect of obesity and risk alleles in the KNG1 gene on VTE risk in a case-cohort study recruited from the general population.

Methods: Cases with a first VTE ($n = 660$) and an age-weighted subcohort ($n = 1793$) sampled from the Tromsø 4 (1994–95) and 6 (2007–08) studies were included. Incident VTEs were registered from baseline to end of 2012, and all VTE events were confirmed by radiological procedures or by autopsy. Obesity was defined as BMI ≥ 30 kg m⁻². Cox regression models were performed to calculate hazard ratios (HR) for VTE in normal-weight and obese subjects with one or two risk alleles at rs710446 in the KNG1 gene in analyses adjusted for age and sex. The regional committees for research ethics approved the study and all subjects gave informed written consent.

Results: The prevalence of one and two risk alleles in the KNG1 gene was 45.7% and 14.1%, respectively, in our study population. Normal-weight (BMI < 25 kg m⁻²) carriers of risk alleles in KNG1 did not have higher VTE risk (HR 1.1, 95CI; 0.8–1.4) than non-carriers. Obese subjects without risk alleles had 1.5-fold (95%CI; 1.1–2.2) higher VTE risk than normal weight subjects without risk alleles. The risk estimates for VTE in obese increased with the number of risk alleles from 1.8 (95%CI; 1.3–2.5) in subjects with one risk allele to 3.4 (95%CI; 2.2–5.2) in those with two risk alleles.

Conclusion: Our findings suggest that the number of risk alleles in KNG1 gene increases the risk of VTE in obese individuals.

Disclosure of Interest: None declared.

PO526-MON

Joint effect of cancer and the Gp6 RS1613662 single nucleotide polymorphism on VTE risk

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Background: Cancer is a risk factor for venous thromboembolism (VTE). The GP6 gene encodes a platelet membrane glycoprotein that acts as a receptor for collagen and plays a key role in platelet pro-coagulant activity and subsequent thrombin and fibrin formation. Pre-cancer platelet count has been previously implicated in VTE in patients with cancer but not in those without.

Aims: To study the joint effect of active cancer and the GP6 rs1613662 single nucleotide polymorphism (SNP) on VTE risk in a case-cohort study recruited from the general population.

Methods: Cases with a first VTE ($n = 616$) and an age-weighted subcohort ($n = 1700$) sampled from the Tromsø 4 (1994–95) and 6 (2007–08) studies were included. Symptomatic VTEs and cancer were registered until the end of 2012. Cox regression was used to determine VTE risk in cancer compared to non-cancer subjects. Only active cancers, meaning 6 months prior to until 1 year following a cancer diagnosis, were designated as cancer. VTE events during this period were defined as cancer-related. The study was approved by the regional research ethics committee and subjects provided informed written consent.

Results: Genotyping was performed and identified 54 individuals with no risk alleles, 567 with 1 risk allele and 1678 with 2 risk alleles at GP6 rs1613662. During follow-up, there were 529 cancer diagnoses and 95 VTEs related to active cancer. The risk of a cancer-related VTE was 9.9-fold (95%CI 7.9–12.4). Individuals with no risk allele at rs1613662 had 17.5-fold (95%CI 6.5–47.1) higher risk of cancer-related VTE. Notably, cancer patients with 1 or 2 risk alleles at rs1613662 SNP had a lower risk of VTE: HR 9.8 (95%CI 6.5–14.9) and HR 8.7 (95%CI 6.7–11.3), respectively.

Conclusion: Our findings demonstrate that individuals with no risk alleles at GP6 rs1613662 are at higher risk of cancer-related VTE than those with 1 or 2 risk alleles. This suggests that both platelet count and platelet aggregation play a key role in VTE in cancer patients, but not in cancer-free subjects.

Disclosure of Interest: None declared.

PO527-MON

Sex hormone-binding globulin and hemostatic variables in women using combined or progestin-only pills

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Background: Combined oral contraceptives (COC) induce a hypercoagulability and increase the risk of venous thromboembolism (VTE). Despite evidence for an important role of activated protein C resistance in COC-induced VTE risk, the underlying mechanisms are not completely understood.

Aims: Using sex hormone binding globulin (SHBG) as a marker for total estrogenicity of pills, we investigated the impact of SHBG on hemostatic variables.

Methods: We conducted a cross-sectional study in 22 progestin-only pill (POP) users, 75 users of several types of COC and 53 non-users. SHBG was measured with an immunometric assay. Thrombin generation-based APC resistance (nAPCr) was measured, together with fragment 1 + 2, D Dimers, free TFPI, tPA and FII (ELISA). Fibrinogen, FVIII, FVII, antithrombin and protein S activities were also measured. Multiple linear regressions were used to assess the relation between SHBG and the hemostatic variables.

Results: Users of COC had the highest SHBG (nmol L⁻¹) mean value compared with non-users and POP users (respectively 90 ± 68, 41 ± 24 and 28 ± 21; $P < 0.01$). In non-user and POP groups, no significant associations between SHBG and hemostatic variables were found. In COC users, SHBG levels were positively associated with nAPCr ($r = 0.36$, $P < 0.01$). Protein S was the only significant mediator and explained about 50% of this association. SHBG levels were also positively associated with factor II levels ($r = 0.35$, $P < 0.01$). Multiple regression showed that both nAPCr and factor II made independent contribution to the prediction of SHBG (partial correlation: 0.34 and 0.36, respectively, $P < 0.01$; $R^2 = 0.23$). There was no other significant hemostatic correlates of SHBG.

Conclusion: While POP have no effect on APCr, COC-related estrogenicity has a major impact on hemostasis. However, changes in APCr remains largely unexplained despite of an important role of protein S. In addition, elevated factor II may be also relevant to VTE risk among COC users.

Disclosure of Interest: None declared.

PO528-MON

Involvement of hereditary thrombophilia in cerebral venous thrombosis occurring in young patients

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Background: Although cerebral venous thrombosis (CVT) is rare, its diagnosis is a challenge for the clinician because of the multiple causes and contributory factors.

Aims: This study aimed to assess the involvement of hereditary thrombophilia in young patients with CVT.

Methods: Thirty three young patients less than 55 years old with CVT were compared to 22 age and sex-matched healthy controls. Demo-

graphic data and risk factors were analyzed. Antithrombin (AT) activity was determined using stachrom AT reagent. Protein C (PC) and protein S (PS) activities were measured by Staclot PC and Staclot PS reagents. The activated protein C resistance test was performed with Staclot aPCR reagent.

Results: The mean age was 36 year-old [23 to 53 year-old]. The sex ratio was 0.1. An underlying disease, a triggering event or a history of fetal loss was observed in 32.35% of cases: post-partum ($n = 5$), pregnancy ($n = 2$), contraceptive pills ($n = 1$), angio-Bechet ($n = 1$), history of fetal loss ($n = 2$). In the other cases, there was no obvious cause. Pregnancy and puerperium-related CVT represented 23.33% of total women. Frequency of congenital anticoagulant factor defects was not significantly different in patients and controls: 9.09% ($n = 3$) in patients and 4.5% ($n = 1$) in controls; $P = 0.6$. There were a PC deficiency in one patient, PS deficiency in one patient and an aPCR related to heterozygous factor V Leiden in one patient and one control.

Conclusion: Hereditary thrombophilia was not a significant risk factor of CVT in young patients. Its interaction with acquired risk factors such as Pregnancy and puerperium may justify a systematic screening for inhibitor markers in CVT.

Disclosure of Interest: None declared.

PO529-MON

Chronic liver disease is a significant risk factor for venous thromboembolism among medical patients in Singapore general hospital

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Background: Chronic liver disease is associated with bleeding risks but increased risks for venous thromboembolism (VTE) were also described in western populations. Information on this is scarce in Asia despite a rising trend of VTE in Asian hospitals.

Aims: To study the prevalence and risks of VTE in medical inpatients with CLD at Singapore General Hospital (SGH).

Methods: The SGH discharge database from 2004 to 2011 was reviewed to identify medical patients with CLD, cancer, venous thromboembolism (VTE) and other comorbid conditions using the Australian Modification (ICD-9-AM) codes in International Statistical Classification of Diseases and Related Health Problems. The prevalence of VTE was analysed with logistic regression analyses and multivariate regression odds ratio (OR) analyses to adjust for age, gender, ethnicity and other co-morbidities.

Results: Of 199904 medical patients, 3.6% (7266) had CLD and 0.9% (1744) had VTE.

Overall prevalence of VTE was significantly increased in CLD patients with 1.5% in non-cirrhosis 2.0% in cirrhosis and 2.5% in liver cancer, compared to 0.8% in patients without CLD ($P < 0.001$).

Similar findings for DVT and PE were seen in non-cirrhosis CLD (DVT-1.0%, PE-0.6%), cirrhosis (DVT-1.7%, PE-0.5%) and liver cancer (DVT-1.6%, PE-1%), compared to non-CLD (DVT-0.7%, PE-0.3%).

Multivariate logistic regression analysis showed that non-cirrhosis CLD (odds ratio [OR] 1.6, 95% CI 1.3–2.1, $P < 0.001$), cirrhosis (OR 1.6, 95% CI 1.1–2.4, $P = 0.020$) and liver cancer (OR 3.2, 95% CI 2.1–4.9, $P < 0.001$) were significant predictors of VTE after adjustment for age, gender, ethnicity, non-liver cancer, infectious disease, and other comorbid conditions such as diabetic mellitus, cardiovascular, cerebrovascular, renal and pulmonary diseases.

Conclusion: Significantly higher prevalence of VTE is seen in our hospitalised medical patients with CLD. The risk further increases with the presence of cirrhosis and cancer. Further studies are required to identify those who will benefit from appropriate preventive measures.

Disclosure of Interest: None declared.

PO530-MON

The effect of hemodiafiltration and hemodialysis on coagulation parameters in patients with end-stage renal disease

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Background: Changes in the hemostatic system might contribute to the highly increased risk of atherothrombotic complications in end-stage renal disease (ESRD).

Aims: Our aim was to test the level of certain hemostatic factors, which might be associated with the risk of thrombosis in these patients.

Methods: Thirty patients being on hemodiafiltration (HDF) treatment for at least one year were enrolled in the study and their hemostasis parameters were measured before as well as 1 and 4 h after the initiation of an actual HDF. Then, their treatment was switched to hemodialysis (HD) for 2 weeks, after which period hemostasis parameters was measured according to the same protocol. Factor VIII (FVIII) activity, antithrombin (AT) activity, and α_2 -plasmin inhibitor activity were determined by chromogenic assays. Factor XIII (FXIII) activity and FXIII-A₂B₂ antigen were measured by ammonia release method and ELISA, respectively. Fibrinogen was determined by Clauss method, immunoturbidimetry was used for the measurement of C-reactive protein (CRP).

Results: FVIII activity was above the reference interval in 46% and 23% of the patients being on HDF and HD treatment, respectively. FVIII levels poorly correlated with CRP concentrations. 26% of HDF patients showed elevated FXIII levels and 17% of them had decreased AT activity. Following HD treatment 43% of the patients had increased FXIII activity. The AT activity was decreased in 27% of the patients. α_2 -plasmin inhibitor activity remained in the reference interval. Fibrinogen levels were slightly/moderately elevated in half of the patients. When the above parameters were corrected for albumin only FXIII activities showed significant elevation during the 4-h course of HDF or HD treatment.

Conclusion: The elevated FVIII and FXIII levels and the decreased AT activity might contribute to the increased atherothrombotic risk of ESRD patients. Evaluation of specific hemostatic parameters in these patients could contribute to a more effective prevention of atherothrombotic events.

Disclosure of Interest: None declared.

PO531-MON

MIR-15A, MIR-96, MIR-144, MIR-150, MIR-155, MI210, MIR-212, MIR-223, MIR-424 AND MIR-451 are differently expressed in platelets from DVT patients

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Background: miRs are small RNAs that regulate negatively the gene expression promoting mRNA degradation.

Aims: We analyzed the miR-15a, miR-96, miR-144, miR-150, miR-155, miR-210, miR-212, miR-223, miR-424 and miR-451 on platelet RNA obtained from 3 spontaneous and recurrent proximal DVT patients and compared to 1 sibling and 1 neighbor for each patient.

Methods: Platelets RNA was obtained, and qPCR reactions were performed using the RT2 *miRNA PCR array* system (Qiagen). U6 was applied as a endogen control.

Results: Results (shown as expression and fold change) were considered significant with fold change higher than 2x or lower than 0.5x. Reduced levels of miRs-96 were observed in patients (0.03 = 1x) in comparison to siblings (0.71 = 21.4x) and neighbors (0.36 = 10.9x), on miR-451 (patients: 0.08 = 1x; siblings: 0.74 = 8.7x; neighbors: 0.36 = 4.3x) and on miR-150 (patients: 0.27 = 1x; siblings: 0.68 = 2.5x), but quite significant in neighbors (0.46 = 1.7x). We observed intermediary expression on miR-15a on patients (0.1 = 1x) in comparison to siblings (0.64 = 6.4x) and controls (0.03 = 0.33x), on miR-210 (patients: 0.18 = 1x; siblings: 0.77 = 4.2x; neighbors: 0.04 = 0.2x) and on miR-155 between patients (0.19 = 1x) and siblings (0.65 = 3.4x), but quite significant in neighbors (0.11 = 0.6). miR-144 was statistically reduced in patients (0.31 = 1x) only in comparison to siblings (0.88 = 2.9x); miR-212 and miR-223 were statistically increased in patients (0.54 = 1x; 0.90 = 1x, respectively) in comparison to neighbors (0.13 = 0.2x; 0.42 = 0.4x, respectively).

Conclusion: miR-15a, miR-96, miR-144, miR-150, miR-155, miR-210, miR-212, miR-223, miR-424 and miR-451 were differently expressed in platelets obtained from DVT patients in comparison to siblings and neighbors. These miRs seems to be related to inflammation (miR-210, miR-155) and to vein thrombosis susceptibility (miR-96, miR-155, miR-223, miR-424, miR-451), and may be related to the thrombotic disease pathophysiology.

Disclosure of Interest: None declared.

PO532-MON

Prevention of venous thrombosis after an anterior cruciate ligament reconstruction: compression stockings alone vs. its combination with low molecular weight heparin

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Background: Anterior cruciate ligament (ACL) reconstruction is one of the most common orthopaedic procedures performed worldwide. Evidence of the effectiveness of venous thrombosis (VT) prophylaxis in these patients is limited and guidelines do not recommend this.

Aims: To establish the effect of low molecular weight heparin (LMWH) in addition to compression stockings after ACL reconstruction on VT prevention.

Methods: We performed an instrumental variable (IV) analysis (a design that mimics a randomised controlled trial) with centre treatment preference as IV (medical ethics committee approved). Data from all patients with ACL reconstruction between April 2011 and June 2013 in clinic A (compression stocking; $n = 441$) and clinic B (compression stocking and LMWH; $n = 936$) were analysed. Both clinics are located in the same geographical region (< 10 km apart) and have the same treatment protocol except for VT prophylaxis. The primary endpoints were VT and bleeding within 3 months after the procedure. To complement the medical records our dataset is being linked to data of the national anticoagulation clinics where all patients with VT are treated (linkage complete at this moment for 826 patients (60%)). Cumulative incidences (CumInc) and risk differences (RD) with 95% confidence intervals (95CI) were calculated.

Results: One patient in clinic A (CumInc 0.23% (95CI: 0.01–1.41)) and 4 patients in clinic B (CumInc 0.43% (95CI: 0.12–1.14)) had a VT, resulting in a RD of 0.20% (95CI: -0.41–0.81). In clinic A, 5 patients had a bleeding event (CumInc 1.13% (95CI: 0.41–2.71)) and 6 patient in clinic B (CumInc 0.64% (95CI: 0.26–1.43)) resulting in a RD of -0.49% (95CI: -1.61–0.62).

Conclusion: The incidence of VT and bleeding in both clinics was low and comparable. An additional protective effect of LMWH for the prevention of VT after ACL reconstruction in patients treated with compression stockings could not be demonstrated.

Disclosure of Interest: None declared.

PO533-MON

Effect of N-homocysteinylation process on tislular plasminogen activator

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Background: Hyperhomocysteinemia proved to be a risk factor for atherothrombosis. Homocysteine thiolactone (HTL) is a highly reactive form that mediates N-binding to ϵ -amino group of lysine residues resulting in modified proteins. Since impair fibrinolysis is associated to thrombosis, changes on N-homocysteinylation tissue plasminogen activator (tPA_{HTL}) properties may be involved in this dysfunction.

Aims: To evaluate HTL *in-vitro* effects on tPA molecule and enzymatic activity.

Methods: Recombinant tPA was incubated with HTL (molar ratio tPA:HTL=1:760; 3 h, 37°C) or saline buffer as control. Samples were dialyzed to remove unreacted HTL. tPA_{HTL} molecule were evaluated by *Capillary Zone Electrophoresis* (CZE). tPA_{HTL} activity was evaluated by a) amyolytic and b) fibrinolytic methods: a) Plasminogen (Plg) activation were studied through kinetic assays with chromogenic substrate S-2251 (0.5 mmol L⁻¹), Plg (0.3 μ mol L⁻¹), tPA_{HTL} vs. tPA_{Control} (360 U mL⁻¹). Sigmoid curves were obtained measuring optical density (OD_{405 nm}) over time. b) Purified fibrinogen (2.5 g L⁻¹) was clotted with thrombin (1 U mL⁻¹) in the presence of Plg and tPA. OD_{405 nm} were recorded vs. time. Maximum lysis velocity (V_{max}) were compared. Assays were performed in sextuplicate.

Results: CZE: electroferograms of tPA_{HTL} showed enhanced migration time vs control (tPA_{HTL} 7.49 $\pm$ 0.08 vs 7.056 $\pm$ 0.009 min). a) Amyolytic assays showed that Plg activation with tPA_{HTL} resulted slower than control (V_{max} HTL 0.0055 $\pm$ 0.0002 vs. 0.0104 $\pm$ 0.0001 min⁻¹); b) Fibrinolysis activity was also diminished by tPA_{HTL} (V_{max} HTL 0.004 $\pm$ 0.001 vs 0.011 $\pm$ 0.002 min⁻¹, $Lysis\ time_{HTL}$ 45.0 $\pm$ 0.7 vs 13.2 $\pm$ 0.4 min). Significant differences ($P < 0.05$) between treated samples and controls were observed in all assays.

Conclusion: N-homocysteinylation induced changes in tPA molecule decreasing its activity. Hyperhomocysteinemic patients would have altered tPA with minor lytic activity, contributing to prothrombotic state.

Subsidio UBA: 20020130100741BA: **Disclosure of Interest:** None declared.

PO534-MON

Increased circulating dna, calprotectin and mieloperoxidase, as neutrophil extracellular trap markers, are risk factors for deep vein thrombosis

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Background: *In vitro*, neutrophil extracellular traps (NETs) are involved in the initiation, growth and resolution of deep vein thrombosis (DVT). DNA, calprotectin (CP) and mieloperoxidase (MPO) are major components of NETs.

Aims: To measure the levels of these 3 circulating NET components in plasma from 193 DVT patients and 198 healthy controls in order to assess the role of NETs on the risk of DVT *in vivo*.

Methods: DNA was measured with PicoGreen (Life Technologies). CP (Hycult Biotech) and MPO (Abnova) were measured by ELISAs. Odds Ratios (ORs) were adjusted for age, sex and the presence of genetic thrombophilic defects.

Results: Patients with DVT had significantly higher levels of circulating DNA (1616 $\pm$ 296 ng mL⁻¹), CP (121 $\pm$ 60) and MPO (2116 $\pm$ 1335) compared with controls (1327 $\pm$ 228, 88 $\pm$ 54 and 1752 $\pm$ 1393, respectively) ($P < 0.001$). There was a significant correlation between the levels of DNA and CP both in cases ($r = 0.245$; $P = 0.003$) and controls ($r = 0.271$, $P = 0.001$) and between CP and MPO in patients ($r = 0.194$, $P = 0.024$). Individuals with levels in the 4th quartile (Q) of controls had a significant increase in the risk of VTE compared with those in the 1st Q: DNA (OR10.7; 95% CI, 3.0–38.3), CP (13.0; 3.5–48.8) and MPO (6.2; 2.1–18.4). Multivariate analysis including the 4th Q of DNA, CP and MPO showed a significantly increased OR only for DNA (15.6; 1.2–203.0).

Conclusion: Increased levels of DNA, CP and MPO were associated with a significant increase in the risk of DVT. The multivariate analysis suggests that the increased VTE risk is via the increase in DNA levels. These results support the potential use of DNase as a new therapeutic tool for DVT prevention or thrombolysis. Funding sources: ISCIII (PI12/00027, RD12/0042/0029, PI14/00512, PI14/00079, FI14/00269), FEDER, Generalitat Valenciana (Prometeo 2015). SN is an IIS La Fe researcher. PM is a Miguel Servet researcher (FIS-CP09/00065).

Disclosure of Interest: None declared.

PO535-MON

In-hospital costs and clinical determinants of costs of acute pulmonary embolism: a prospective study

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Background: Pulmonary embolism (PE) is a frequent and severe disease. It is also an important driver of costs particularly during the

acute – usually in-hospital – phase of its management. Available medico-economic studies are retrospective and were mostly conducted in North America. Clinical predictors of costs have not yet been assessed
Aims: To prospectively assess in-hospital costs and initial clinical predictors of costs of acute PE in France

Methods: Prospective, monocentre, observational medico-economic study conducted in Montpellier University Hospital. From May 2012 to May 2013, all patients hospitalized or admitted to the emergency room for an objectively confirmed PE were included. Hospital-acquired PE patients were excluded. Patients' demographics, social and clinical characteristics and outcomes were reported. In-hospital costs were estimated from the French hospital discharge database. We assessed the influence of demographics and clinical characteristics of patients at baseline and initial severity of PE on these costs

Results: Two-hundred and three patients were included. Patients' mean age was 68 years and 27% had a Pulmonary Embolism Severity Index risk class of I or II. In-hospital mortality was 8.5%. Mean length of hospital stay was 12 days and mean total hospital cost per patient was 5301€ or \$6043

Initial severity of PE (severe or intermediate vs. non-severe, $P = 0.003$), presence of an active cancer ($P = 0.026$), a chronic heart failure ($P = 0.014$) or a severe renal impairment ($P = 0.035$) independently increased costs. Age, sex, altered mental status, chronic respiratory disease, recent/risk of bleeding, haemoglobin and platelet levels, occurrence of PE while on anticoagulants had no significant impact on these costs

Conclusion: In this French study, in-hospital costs for acute PE were substantial but lower than those usually reported in North American studies (\$14,000). Initial severity of PE, presence of an active cancer, of a severe renal impairment or of a chronic heart failure independently increased these costs

Disclosure of Interest: None Declared.

PO536-MON

Identification and validation of plasma micrornas involved in venous and arterial thrombosis

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Background: Circulating microRNAs (miRNAs) are detectable in plasma, requires non-invasive sampling, and have been described as promising clinical biomarkers.

Aims: To identify and validate the miRNAs present in the plasma of patients with venous thrombosis without (VT) and with recurrence (reVT), and myocardial infarction (MI).

Methods: We analyzed the miRNA expression in 6 plasma samples from VT patients, 6 with reVT, 6 with MI, and 6 healthy subjects using the GeneChip miRNA 3.0 Array Platform (Affymetrix). The results were analyzed using the PARTEK Genomic Suite software, and confirmed by RT-qPCR in 100 samples (20 samples of each subgroup).

Results: The analysis of the miRNA expression separated VT, reVT and MI samples from controls. We selected 24 miRNAs which expression in VT, reVT and IM were dysregulated compared to controls ($P < 0.05$ and $FD > 1.5$). RT-qPCR confirmed the different expression in 6 miRNAs. Compared to controls, the expression of miR-106b and miR-126-3p were 33% and 81% increased in MI patients ($P = 0.029$ and $P < 0.001$ respectively), miR-122 was 248% and 360% increased in reVT and MI patients ($P = 0.044$ and $P < 0.001$ respectively), miR-140-3p was 54% and 82% increased in VT and MI patients ($P = 0.039$ and $P < 0.001$ respectively), miR-744-5p was 61% increased in reVT

($P = 0.032$), and let-7e was 43% reduced in VT ($P = 0.002$). Computational prediction of miRNA targets using miRWalk tools showed that these miRNAs interact with proteins involved in the coagulation, leucocyte transendothelial migration or lipid metabolism pathway ($P < 0.05$).

Conclusion: We identify, for the first time, a miRNA profile characteristic of VT and reVT. Further studies are needed to determine the functional consequences of this imbalance and their use as diagnostic tools. ISCH (PI12/00027, RD12/0042/0029, PI14/00512, PI14/00079, FI14/00269, FIS-CP09/00065), FEDER, Generalitat Valenciana (Prometeo 2015), IIS La Fe (2012/0221).

Disclosure of Interest: None Declared.

PO537-MON

The incidence of catheter-related thrombosis in patients on long-term parenteral nutrition: a systematic review and meta-analysis

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Background: Long-term parenteral nutrition (PN) is indicated in patients with severe intestinal failure. Patients on PN require a central venous access, which increases the risk of central venous catheter-related thrombosis (CRT), ultimately leading to loss of vascular access and intestinal transplantation.

Aims: The aim of this systematic review is to assess the incidence of CRT in adults on long-term PN.

Methods: We systematically searched Medline and Embase for prospective or retrospective studies including at least 25 patients on PN and an adequate follow-up length (> 3 months). Only studies published in the past 15 years were included, as low-molecular-weight heparins, new generations of central venous catheters and new PN feeding formulations have become available since then. Primary outcomes were the incidence rates of symptomatic objectively- and non-objectively-diagnosed CRT. The meta-analysis was performed by calculating a weighted mean of the rates: the weight was determined by the size of the study population, expressed as patient-years (pt-yrs).

Results: We identified 2379 studies, of which 7 were included. We calculated an incidence rate of 2.4/100 pt-yrs (95%CI 1.3–3.5) for objectively-diagnosed symptomatic CRT on the basis of 4 studies and an incidence rate of 7.9/100 pt-yrs (95CI: 2.1–11.8) for non-objectively-diagnosed CRT. Anticoagulation administration was not mentioned in any of the included studies.

Conclusion: The incidence of CRT in patients on PN occurs at a rate higher than 2% per pt-yr. CRT rate has been poorly documented for the past 15 years. The higher rates of non-objectively diagnosed CRT could lead to unnecessary anticoagulation and concomitant increased risk of bleeding. Well designed intervention studies evaluating the efficacy and safety of anticoagulant prophylaxis are needed.

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PO538-MON

Vena cava thrombosis in adulthood vs pediatrics patients: different phenotypes and risk factors for the same entity

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Background: The vena cava thrombotic phenomenon is a rare condition with wide clinical presentation and characteristics.

Aims: To investigate the clinical presentation and treatment of patients with vena cava thrombosis (VCT) at diagnosis in a tertiary referral hospital in Barcelona Spain.

Methods: A retrospective analysis was performed of all newly diagnosed patients with vena cava thrombosis (VCT) at the haemostasis unit from January 2007 to February 2014.

Results: Ninety-five patients with VCT were recruited, of whom 55 were pediatrics patients and 43 adults. The median age at diagnosis within the pediatric patients was 4 months (range: 1–192 days) with higher male incidence (63.5%). Inferior vena cava (IVC) thrombosis within the suparenal compartment was the most frequent event representing a 61.5% while superior vena cava (SVC) and the IVC with infrarenal involvement represented a 23% and 15.4% respectively. A 35.5% of these cases were diagnosed by echocardiogram and were frequently associated with congenital heart surgery while the SVC thrombosis was catheter related in 58%. 90.6% of the patients received low-molecular-weight heparin (LMWH) with treatment duration of three months.

In adults the median age at presentation was 51 years (range: 18–84) also with a higher male incidence (58.1%). SCV thrombosis was presented in 53.4% of patients. Most of them were oncological patients (65.2%) while only 45% were catheter related. LMWH treatment was used in a 90% of cases for at least 6 months. Thrombophilia was performed in 30 cases and was present in 9.5%.

Conclusion: The retrospective analysis of the cohort showed two clear different phenotypes and different risk factors depending on the age at presentation. The IVC thrombosis was frequent in the pediatric patients, associated with congenital heart diseases while the SCV was the most common site in adulthood, usually associated with neoplasm malignancies. Further studies are needed to determine clearly the risk factors and those patients who can benefit of thromboprophylaxis.

Disclosure of Interest: None declared.

Thrombophilia I

PO539-MON

A clinical audit of heritable thrombophilia test profile in a tertiary health care facility, Pakistan

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Background: Heritable thrombophilia is evaluated by performing a range of screening and diagnostic test. However, the effectiveness of such evaluations is largely determined by limiting improper investigations, either in inappropriate patients or at unsuitable time points.

Aims: To review clinical ordering patterns of requests for heritable thrombophilia and to audit these findings against an international standard.

Methods: Review of requests for heritable thrombophilia profile was conducted from January-June 2014. Data for patient characteristics, clinical indication, provoked/unprovoked event, time of performing

test and cost incurred were obtained. The 2010 British Committee for Standards in Haematology (BCSH) clinical guidelines for testing for heritable thrombophilia was used as the standard for the audit.

Results: On 103 patients 118 requests were made. Median age was 35 years. Fifty five (47%) tests were abnormal of which only 15 (27%) were repeated and only 2 (2%) of all patients were confirmed with a heritable thrombophilia. Indications for testing were deep vein thrombosis 37 (36%), stroke 32 (31%), intra-abdominal vein thrombosis 18 (18%), arterial thrombosis 4 (4%) and pulmonary embolism 2 (2%). Ten percent of patients were screened only on clinical suspicion though they did not show validated thrombosis radiologically. Provoked thrombosis occurred in 24 (23%) patients. Ninety six (93%) tests were performed on patients on anticoagulation and during acute thrombotic event. Total cost incurred on 118 tests was around \$29500. Patients tested appropriately for time and clinical indications were 7 (7%) and 44 (43%) respectively. Five percent of patients were tested appropriately with respect to both clinical indications and test timing hence justifying 5% of the cost expenditure.

Conclusion: The large majority of requests did not satisfy the BCSH criteria. Poor patient and timing selection compromised the utility of these tests and led to increased wastage of health care cost.

Disclosure of Interest: None declared.

PO540-MON

Thrombophilic profile in extrahepatic portal vein obstruction- experience from a tertiary centre in India

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Background: In India, extrahepatic portal vein obstruction (EHPVO) is responsible for about one third cases of adults and more than half of the cases in children as a cause of portal hypertension. Role of various thrombophilic markers and treatment strategies are not clearly established.

Aims: To study the thrombophilic profile, treatment and outcome of patients with EHPVO.

Methods: A retrospective study of patients with EHPVO who visited the tertiary care centre was conducted. Characteristics of clinical presentation, etiology of EHPVO, management and outcome were analyzed.

Results: A total of sixty seven cases were diagnosed with EHPVO during last 2 years. Out of these, 45 were males and 22 were females. Mean age of presentation was 31.9 ± 13.9 years. Clinically, most of the patients 32 (48%) presented with pain abdomen followed by upper GI bleeding in 18 (26.8%) cases. Thrombophilia work up done showed risk factors in 44 (65.7%) cases which include Protein S deficiency in 24 cases mean value (36.3 ± 13.07), Protein C deficiency in 2 cases, homozygous methyltetrahydrofolate reductase in 3 cases, homozygous JAK2V617F mutation in one case and hyperhomocysteinemia in 16 patients (8 cases had associated Protein S deficiency). Work up for antiphospholipid syndrome was done in 46 cases out of which three patients showed significant antiphospholipid and anticardiolipin Ig M levels. One patient was diagnosed as immune thrombocytopenia and one patient was a diagnosed case of sickle cell trait. None of the patients was positive for Prothrombin G20210A mutation and one patient was heterozygous for Factor V Leiden.

Treatment: Shunt operations done in five patients because of uncontrolled bleeding. Anticoagulation was started in cases of acute thrombosis and duration was decided with regard to risk to benefit ratio in each case.

Conclusion: This study concludes that concurrence of prothrombotic disorders is more common than expected and studies are needed to guide the duration of anticoagulation.

Disclosure of Interest: None Declared.

PO541-MON

ISTH's clarion call: 'to raise the awareness of thrombosis worldwide – a global disease burden'Ayadurai T¹, Faridah AM² and Karim FA²¹Haemostasis Laboratory, Faculty Of Medicine, University Malaysia Sabah, Malaysia., Kota Kinabalu; ²Haemostasis Laboratory, National Blood Centre, Kuala Lumpur, Malaysia

Background: To raise the awareness of thrombosis worldwide, the International Society on Thrombosis & Haemostasis (ISTH) has dedicated 13th October as the World Thrombosis Day (*J Thromb Haemost* 2014; 12: 1579). This annual event would accord venous thromboembolism (VTE) its due clinical importance when VTE failed to be reported in the Global Burden of Diseases, Injuries and Risk Factors Study 2010.

Aims: To answer the clarion call of ISTH, thrombosis patients were analysed over a period of 1 year 2014 to determine the prevalence of thrombosis in the patient-population of a city-based hospital in Kuala Lumpur, Malaysia, a developing Asian country.

Methods: Four-hundred thrombosis patients aged 30 to 60 years (254 females + 146 males; ethnicity: 255 Malays, 39 Chinese, 106 Indians) were categorized into arterial or venous and deep vein thrombosis (DVT), pulmonary embolism (PE), stroke and other-VTE (e.g. mesenteric, portal, cephalic thrombosis).

Results: Venous thrombosis was identified in 311 (77.8%) patients and arterial in 89 (22.2%) of which 254 (63.5%) were females and 146 (36.5%) males. There were 133 DVT (33.25%), 120 other-VTE (30.0%), 89 strokes (22.25%) and 58 PE (14.5%). In the Malays there were: 39.2% DVT, 25.9% other-VTE, 19.2% PE and 15.7% stroke. In the Chinese it was 38.5% other-VTE, 33.3% DVT, 20.5% stroke and 7.7% PE. The Indians showed 38.7% stroke, 36.8% other-VTE, 18.9% DVT and 5.7% PE.

Conclusion: Venous thrombosis was more prevalent than arterial by an overwhelming factor of 3.5 to 1.0 ($P < 0.05$) with DVT being the commonest and PE the least. Females exceeded males in the incidence by a ratio of 1.7:1.0. The prevalence of thrombosis in the ethnicities was 62, 35 and 5 per 1000 in the Indians, Malays and Chinese respectively. The most common thrombosis event in the Malays was DVT (39.2%), strokes in the Indians (38.7%) and other-VTE (38.5%) in the Chinese ($P < 0.0001$). The healthcare authorities could use these data to formulate relevant policies and resources to face this serious disorder.

Disclosure of Interest: None declared.

PO542-MON

Risk factors of thrombosis and sickle cell anemia

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Background: Deep vein thrombosis is a common risk among people with sickle cell disease or trait. Stroke, low limb thrombosis and acute chest syndrome occur more frequently

Aims: Study thrombosis risk factors in patients with sickle cell SS or AS compared with healthy AA people

Methods: We led an analytical case-control study during 24 months, from January 2012 to February 2014. All consenting patients had been sent to the laboratory by clinical hematologists.

Sickle cell patients SS and AS were matched by age and sex to AA. All have benefited from a standard hemostasis tests (PT, APTT, Fib), hemogram, blood grouping and dosage of (PS, PC, AT, and antiphospholipids (aCL, aB2GPI, LA). Analysis of the results was performed using SPSS 20.0 software.

Results: The average age of our population was 26.52 years, ranging from 13 to 42 years and the sex ratio was 0.56.

PS deficiency was found in 54% ($n = 27$) of the SS sickle cell disease, 14% ($n = 7$) AS sickle cell disease and 20% ($n = 10$) of healthy people. The lupus anticoagulant was found in 32% ($n = 16$) of the SS and AS SCD and 10% ($n = 5$) of control group.

The presence of anti-β2GPI was also found in 36% ($n = 18$) SS sickle cell, 6% ($n = 3$) of AS and 2% ($n = 1$) of healthy people.

These hematological risk factors were found significantly associated with the presence of HbS unlike the ABO blood group.

These hematological risk factors were found significantly associated with the presence of HbS unlike the ABO blood group.

Conclusion: The prevalence of PS and PC deficiency and the presence of antiphospholipid antibodies in the patient can not explain all thrombotic events found in homozygotes. However, the study shows a significant association between the occurrence of stroke, acute chest syndrome, of deep vein thrombosis of limb.

Disclosure of Interest: None Declared.

PO543-MON

Genetic variability of *KNG1* and *F11* genes using next generation sequencingMartin-Fernandez L¹, Corrales I², Ramirez L², Martinez-Perez A¹, Brunel H¹, Souto JC³, Vidal F² and Soria JM¹¹Unit of Genomics of Complex Diseases, Research Institute of Biomedicine (IIB-Sant Pau); ²Unit of Molecular Diagnosis and Therapy, Blood and Tissue Bank; ³Unit of Hemostasis and Thrombosis, Department of Hematology, IIB-Sant Pau, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

Background: Venous thromboembolism (VTE) is a complex disease with a high heritability. However, only a portion of the genetic component is accounted for by genetic risk factors. From the GWAS of factor XI (FXI) performed in the GAIT-1 (Genetic Analysis of Idiopathic Thrombophilia) project significant associations were identified at *KNG1* and *F11* loci.

Aims: We performed Next Generation Sequencing (NGS) of the *KNG1* and *F11* genes to identify genetic variants that might be involved in the risk of thrombosis.

Methods: We studied 40 unrelated individuals from the GAIT-2 project, which consisted of 935 individuals in 35 extended families selected through a proband with idiopathic thrombophilia. We amplified 60,052 bp encompassing the *KNG1* and *F11* loci. Sequencing libraries were prepared using the Nextera XT DNA Sample Preparation kit (Illumina, USA) which ran on an Illumina MiSeq system. Bioinformatic softwares were used to analyse the sequencing data. Variants were compared to the variants that were genotyped in the GWAS of the GAIT-2 project (HumanOmniExpressExome-8v1.2 with 964,193 variants) and filtered to identify functional mutations.

Results: Using a median coverage of 205x, 98.5% of *KNG1* and *F11* were sequenced. A total of 1180 variants were detected and 60.2% of them were new (not included in the GAIT-2 GWAS panel). There were 8 target variations selected for *in-silico* predictions (1 missense, 1 3'UTR, 2 intronic and 4 upstream variants).

Conclusion: We observed that most of the variants were not included in the GWAS panel. Therefore, it is clear that GWAS is limited as compared to NGS which is a method that might help to fill up the gap of the 'missing heritability'. These variants probably play a role in thrombotic risk. In the future, we will genotype all of the variants that we found in the entire GAIT-2 sample in hopes of identifying their contribution to the plasma FXI levels and the risk of thrombotic disease.

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Disclosure of Interest: None Declared.

PO544-MON

Laboratory markers of thrombophilia

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Background: Thrombophilia means increased tendency to develop venous or arterial thrombosis. This condition may be inherited or acquired. In inherited, thrombotic complications are usually in the venous circulation. Inherited thrombophilic defects include ATIII, PC and PS deficiencies, activated PC resistance (APCR), factor V Leiden (fVL), prothrombin (II G20210A) variant etc.

Aims: *In vitro* detect a hypercoagulability by specific laboratory methods.

Methods: One-hundred patients with a history of DVT, PE and AIM. Biological activity of ATIII, PC, PS with Simens BERICHROM ATIII and PC, PS Ac kits and resistance to activated PC (ProC[®] Ac R) were measured on BCS-XP. According to reference values ATIII, PC and PS activity <60% were regarded as deficiency. A ratio of activated PC <2.1 indicated APCR. fVL and II G20210A polymorphism were detected with PCR specific polymorphism detection kits (Biosystems) on a ABI Prism 7000 equipment.

Results: Twenty-nine patients from 100 had APCR (1.64 ± 0.22 vs. 2.24 ± 0.51). 15 patients from all had decreased level of PC ($40.13\% \pm 18.36$ vs. $101.47\% \pm 39.91$) and 19 patients had decreased level of PS ($43.11\% \pm 12.93$ vs. $89.6\% \pm 39.68$). All examined had normal ATIII ($96.82\% \pm 16.96$). 15 patients were heterozygotes and one was homozygote for fVL. 9 patients were heterozygotes for II G20210A. One patient with AIM was heterozygote for fVL and II, and one patient with Phlebotrombosis was homozygote for fVL and heterozygote for II. This patients were APCR (1.51 ± 0.44). 14 patients with heterozygote fVL were at same time APCR (1.68 ± 0.16).

Conclusion: Recognition of thrombophilic defects (decreased ATIII, PC, PS, APCR) has changed the diagnostic and therapeutic approach to patients and has had an important influence on laboratory screening of family members. Inherited thrombophilia (fVL and II G20210A polymorphism) is genetically determined high predisposition to develop thromboembolic complications. The availability of new lab tests for thrombophilia has opened a new era for its diagnostic, prevention and prophylaxis.

Disclosure of Interest: None Declared.

PO545-MON

Evaluation of protein S specific activity permits accurate diagnosis of protein S tokushima, a missense mutation with type II protein S deficiencyTsuda H^{1,2}, Noguchi K¹, Nakazono E², Tsuda T³ and Jin X³

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Background: Protein S (PS) Tokushima, p.Lys196Glu (rs121918474), is a genetic risk factor for venous thromboembolism in a Japanese population with the frequency of heterozygotes about 2%. Its phenotype of type II deficiency makes it difficult to screen PS Tokushima by using conventional plasma PS assays. We have developed a new total PS-assay system (Tsuda, T et al., Blood Coagul Fibrinolysis. 56, 2012) to determine the specific activity of plasma PS.

Aims: We investigated whether the evaluation of PS specific activity is sensitive enough to identify the carrier of PS Tokushima mutation.

Methods: Young Japanese healthy women ($n = 143$, 20.5 ± 0.4 years old) were recruited. The activity and protein levels of total PS in plasma were measured using the total PS-assay system, and then the

specific activity (activity/protein levels) was calculated. PS Tokushima was determined either by PCR-RFLP analysis or real-time PCR using cycling-probe (Takara-Bio). The study was approved by the ethics committee of Nakamura Gakuen University and the all participants gave informed consent.

Results: Four subjects (2.8%) were found to be heterozygous carriers of PS Tokushima mutation. The PS specific activity levels of heterozygotes ($n = 4$, mean $\pm$ SD 0.69 ± 0.04 , minimum-maximum range $0.66-0.74$) were significantly lower than wild-type ($n = 139$, 0.99 ± 0.07 , $0.78-1.17$, $P < 0.0001$), permitting a definitive diagnosis of PS Tokushima. Although the total PS activity levels of heterozygotes (16.6 ± 1.5 , $15.7-18.8 \mu\text{g mL}^{-1}$ PS equivalent) were significantly lower than wild-type (23.5 ± 3.6 , $16.4-33.3 \mu\text{g mL}^{-1}$ PS equivalent, $P = 0.001$), the data distributions of two groups overlapped one another. No difference in total PS protein levels was found between two groups (heterozygotes $24.2 \pm 2.7 \mu\text{g mL}^{-1}$, wild-type $23.8 \pm 2.7 \mu\text{g mL}^{-1}$, $P = 0.775$).

Conclusion: The heterozygous carriers of PS Tokushima mutation are certainly identified by assessment of PS specific activity even in the case of young women, whose plasma PS levels are decreased compared with men and post-menopausal women.

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PO546-MON

Hypoxia induces a prothrombotic state independently of the physical activityNinivaggi M^{1,2}, de Laat M¹, Lancé M³, Kicken C^{1,3}, Peters T^{1,2}, Bloemen S^{1,2}, Dirks M⁴, van Loon L⁴, Govers-Riemsag J², Krishnamoorthy G¹, Lindhout T¹, Konings J^{1,2} and de Laat B^{1,2}

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Background: Studies investigating the role of hypoxia on coagulation, by either going to high altitude or after a long-haul flight, reveal contrasting results. However, mostly a prothrombotic phenotype is found. **Aims:** We attempted to get a better comprehension of this mechanism by going into the Swiss Alps, thereby including the potential contributing role of physical activity.

Methods: Two groups of 15 healthy individuals were formed that suffered from hypoxia due to an increase in altitude up to 3900 m. Group A ascended actively by climbing and group B was transported passively. Heart rate, oxygen saturation levels, blood pressure, factor (F) VIII and Von Willebrand Factor (VWF) levels as well as thrombin generating (TG) capacity in plasma and whole blood (WB-TG) were measured. As a control for the passive group, 7 healthy donors stayed immobile in bed for 7 days at normoxic condition.

Results: Heart rate increased and oxygen saturation levels decreased with increasing altitudes, but blood pressure was not affected. FVIII and VWF levels increased significantly only in the active group. In contrast to the passive group, plasma TG of the active group increased, which could be explained by elevated VWF/FVIII levels. WB-TG revealed that hypoxia was associated with a faster and higher TG in both groups. This would indicate that the cellular portion of the blood is at least partially responsible for the association between hypoxia and venous thrombosis. Blood samples collected at 3900 m, but measured at 3900 m and 1000 m, showed aberrant results. Therefore, processing and measurements of the samples should occur at the same altitude as the blood drawing. Immobility for 7 days in bed did not affect coagulation parameters.

Conclusion: By applying WB-TG we demonstrated that hypoxia causes a prothrombotic state. The results between plasma and WB-TG differ,

suggesting that the cellular part of the blood is involved in the prothrombotic phenotype, independent of the physical activity.

Disclosure of Interest: None Declared.

PO547-MON

The possible role of c.1824C>T prothrombin gene variant in pathogenesis of thrombophilia

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Background: Prothrombin (FII) is a precursor of thrombin, which represents one of the key haemostatic enzymes. Elevated level of prothrombin leads to hypercoagulation and thrombosis. The 3' end of the prothrombin gene has noncanonical architecture that is sensitive to gain-of-function mutations, leading to increased prothrombin expression and elevated risk for thrombophilia. The novel FII c.1824C>T gene variant is located in the last exon of the prothrombin gene and it has not been associated with thrombophilia, so far.

Aims: In order to examine the possible role of FII c.1824C>T variant in pathogenesis of thrombophilia we performed case-control study and determined the plasma prothrombin level in carriers of this gene variant.

Methods: Our study was carried out in a group of 443 patients with thrombotic events (38 ± 13.84y; 223 m/220f) and 191 healthy subjects (38 ± 10.31y; 85 m/106f). The FII c.1824C>T gene variant was detected by polymerase chain reaction, followed by digestion with specific restriction enzyme or by direct DNA sequencing. The plasma level of prothrombin was detected by Western blot analysis using method of relative quantification.

Results: We observed higher frequency of heterozygous carriers of FII c.1824C>T gene variant in the patient group compared to the controls (3.39% vs. 0.52%, $P = 0.049$). Homozygous carriers were not detected. According to our results, presence of this variant leads to the increase of prothrombin level in the plasma of heterozygous carriers. Compared to the standard plasma (reference value 100) the amount of prothrombin in the carriers plasma was 143.65 ± 28.8 ($P < 0.05$).

Conclusion: Our results indicate that FII c.1824C>T gene variant may be a potentially important thrombophilia marker.

Disclosure of Interest: None Declared.

PO548-MON

Pathogenetic prophylaxis pre-eclampsia in patients with thrombophilia and the history of pre-eclampsia

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Background: In this article we are talking about the effectiveness of antithrombotic prevention of recurrent preeclampsia in women with thrombophilia.

Aims: Evaluation of pathogenetic prophylaxis of recurrent pre-eclampsia in patients with thrombophilia and pre-eclampsia in anamnesis.

Methods: I group: 45 (52.9%) patients with PE in anamnesis – prophylaxis treatment was initiated since the fertile cycle and during pregnancy.

II group: 40 (47.1%) patients with PE in anamnesis – prophylaxis treatment was initiated during pregnancy (from 6 to 13 weeks of gestation). Control group – 70 women with noncomplicated pregnancy.

Results: IUGR was in 9 patients – 3 patients -I group and 6 – II group. PE moderate and severe was prevented, mild PE occurred in 3 patients of the II group; in the I group and control was no cases. Preterm caesarean section was not in the I group and control group; in the II group in 2 (5%) cases.

Preventive therapy include: LMWH (Enoxaparin 20–60 mg), antioxidants, vitamin B, folic acid, aspirin (except the I trimester and lactation). Patients with APA received natural progesterone. LMWH was discontinued 24 h prior caesarean section and resumed after 6–8 h after birth.

In all cases was performed caesarian section by obstetrician reasons, in control group in 17 (24.3%) cases.

Alive and healthy children were born in all of women with history PE (100%).

Conclusion: Patients with PE in anamnesis should be investigated for thrombophilia. Patients with thrombophilia require an early start of pathogenetic prophylaxis therapy. Preventive treatment with LMWH is highly effective for the prophylaxis of recurrent pre-eclampsia.

Disclosure of Interest: None declared.

PO549-MON

The clinical and laboratory analysis of homozygous carriers factor V leiden mutation

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Background: Factor V Leiden (FVL) is inherited mutation associated with an increased risk for venous thromboembolism (VTE).

Aims: Retrospective clinical and laboratory characterization of homozygous FVL carriers diagnosed and treated in the period 1995–2014.

Methods: 2495 heterozygous carriers of FVL mutations and 96 homozygous cases were admitted for diagnosis. In 5/96 homozygous patients, FVL occurred together with heterozygous G20210A mutation of prothrombin gene. We analyzed 67 homozygous FVL mutation patients (41F; 26M, age 18–64).

DNA was isolated using GeneMatrix kit (Eurz, Poland). The FVL mutation was identified by PCR/RFLP using restriction enzyme MnlI (Eurz, Poland). Prothrombin G20210A mutation was determined using Hind III restriction enzyme (Eurz, Poland).

Results: 1. Homozygous FVL was detected in 41 female (61.2%) and 26 male (38.8%) patients. Deep venous thrombosis (DVT) was observed in 60/67 patients.

2. Mean age of female and male patients was similar (30.5 vs. 30.7 yrs) at the moment of the first DVT episode.

3. Proximal and distal DVT was more often observed in female than male patients.

4. 2–4 episodes of DVT occurred in 8/60 pts (13.3%) more often in female patients.

5. All the patients had several thrombotic risk factors: 6. Mean age of female asymptomatic FVL patients was higher (41.0 yrs) than male patients (29.3 yrs) and the dominant thrombotic risk factors were familiar DVT.

7. Treatment: low molecular weight heparin followed by anticoagulant vitamin K.

Conclusion: In carriers of homozygous FVL mutation additional pathophysiological, environmental and genetic thrombotic risk factors are necessary for venous thromboembolism to occur.

Disclosure of Interest: None Declared.

PO550-MON

Molecular characterization of new antithrombin mutations

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Background: Antithrombin (AT) deficiency is classified as type I (quantitative) and type II (qualitative) deficiency. More than 230 mutations have been described in the gene encoding AT, so far.

Aims: To describe the mutation spectrum of AT deficiency in Hungary and to characterize novel mutations at molecular level.

Methods: Non-related AT deficient individuals ($n = 82$) were genotyped for causative mutations within AT gene (SERPINC1) and novel mutations ($n = 6$) were selected. Wild type and mutant plasmids were transfected to HEK293 cells and the expressed AT were investigated by ELISA and Western blotting (WB). Quantitative determination of mRNA was executed by qPCR. Intracellular localization of AT was examined by immunofluorescent staining detected by confocal laser scanning microscopy. Structural alterations were investigated by molecular modeling.

Results: The following mutations were found: p.Leu205Pro, p.Asn450Ile, p.Gly456delins_Ala-Thr, p.Pro461Thr, p.Leu270ArgfsX13 and dupc.315–334. The 205Pro AT was detected intracellularly in the same level as wild type, however a tiny amount of mutant AT was secreted into the medium. This mutant showed strong co-localization with the 26S proteasome. *In silico* experiments suggested major structural alteration. The levels of Ile450, 456Ala-Thr and 270ArgfsX13 AT mutants were strongly reduced in the cell lysates and no AT was detected in the cell media. The 461Thr mutant was detected in normal amount both in the cells and in the media. mRNA level was decreased in the case of 456delinsAla-Thr and 270ArgfsX13 and it was normal for 450Ile.

Conclusion: The p.Leu205Pro mutation leads to impaired folding and secretion defect; the mutant AT retains in the 26S proteasome and suffers intracellular degradation. Defective mRNA synthesis or degradation leads to AT deficiency in case of the delins and frameshift mutations. Altered protein synthesis or rapid protein degradation for 450Ile AT and a functional defect for 461Thr AT are suggested.

Disclosure of Interest: None declared.

PO551-MON

A reason to include homocysteine in the hypercoagulable workup

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Background: Hyperhomocysteinemia has been linked with thrombosis but it is unclear as whether it is causative or is an epiphenomenon.

Checking homocysteine as part of a hypercoagulable workup is not recommended. Hyperhomocysteinemia is also a manifestation of vitamin B12 deficiency, which has been associated with thrombosis in case-control studies. Evaluation for hyperhomocysteinemia in patients with thrombosis could lead to the diagnosis of vitamin B12 deficiency.

Aims: To determine the detection rate of vitamin B12 deficiency in patients with thrombosis screened for hyperhomocysteinemia.

Methods: Institutional Review Board approval was obtained. The charts of all patients with a history of thrombosis referred to a hematology practice for a thrombophilia evaluation between 01/01/14 and 12/31/14 were reviewed. Homocysteine levels (usually fasting) had been checked in all patients, and in those patients with hyperhomocysteinemia, methylmalonic acid levels had been checked.

Results: Forty-seven patients met criteria for enrollment in this study. Twenty-six patients had hyperhomocysteinemia: mean homocysteine level $22.09 \mu\text{mol L}^{-1}$, range $16.1\text{--}32.3 \mu\text{mol L}^{-1}$ (normal range $5\text{--}15 \mu\text{mol L}^{-1}$), and 20 of these patients were evaluated further for methylmalonic acidemia. Methylmalonic acid levels were elevated in 2 patients at 610 and 640 nmol L^{-1} (normal range $87\text{--}318 \text{ nmol L}^{-1}$); fasting homocysteine levels in these patients were 23 and $20.5 \mu\text{mol L}^{-1}$, respectively. Both patients were started on vitamin B12 $1000 \mu\text{g PO}$ daily.

Conclusion: The detection rate of vitamin B12 deficiency in this cohort was 4.2% . The two patients identified were started on high-dose vitamin B12 supplementation. While it is unclear if high-dose vitamin B12 therapy prevents recurrent thrombosis, it would prevent progressive vitamin B12 deficiency. Further studies should examine the relationship between vitamin B12 deficiency and thrombosis.

Disclosure of Interest: Bone declared.

Tissue factor – Factor VII – I

PO552-MON

Pathological determinants of human coronary thrombus formation: distinct procoagulant activity and lactate production of M1/M2 polarized macrophages

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Background: Large thrombus formation on disrupted atherosclerotic plaque is a critical event that leads to the onset of acute myocardial infarction (AMI). We recently reported that arterial glucose metabolism might reflect vascular thrombogenicity.

Aims: This study aimed to determine vascular factors involved in thrombus formation that leads to AMI onset, and procoagulant activity and glycolysis in M1/M2 polarized macrophages.

Methods: This study was approved by the institution ethics committee. We histologically and immunohistochemically analyzed culprit lesions in 40 coronary arteries with thrombi at autopsy after lethal AMI and non-cardiac death (asymptomatic plaque disruption). Tumor necrosis factor- α and interferon- γ polarized cultured THP-1 macrophages to the M1 phenotype or interleukin-4 and -13 polarized them to the M2 phenotype.

Results: Thrombus size and the ratios of thrombi to luminal areas were significantly larger in coronary arteries with AMI than with asymptomatic thrombus. Areas that were immunopositive for tissue factor (TF) and cells that were positive for hexokinase (HK)-II and glucose transporter-1 were abundant in plaques of AMI. The size of coronary thrombus positively correlated with the length of plaque disruption ($r = 0.80$) and with areas that were immunopositive for TF ($r = 0.38$) and HK-II ($r = 0.40$). Both M1 and M2 macrophages expressed TF and HK-II in symptomatic plaques. The expression of TF and proco-

agulant activity were increased and that of HK-II and lactate production were decreased in M2-, compared with M1-polarized macrophages. Inhibiting glycolysis enhanced TF expression and activity in the macrophages.

Conclusion: The degree of plaque disruption and expression of TF and HK-II appear to be important vascular factors for AMI onset. Our results suggest a link of glucose metabolism to procoagulant activity in the polarized macrophages and distinct contribution to thrombogenicity and glucose metabolism.

Disclosure of Interest: None declared.

PO553-MON

Single phosphorylation of the cytoplasmic domain of tissue factor at serine 253 is sufficient for the binding of tissue factor to filamin-A

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Background: The cytoplasmic domain of tissue factor (TF) contains two serine residues (253 and 258) which are phosphorylated. Previously it was reported that double-phosphorylation of TF enhances its interaction with the C-terminus repeats 22–24 of the cytoskeletal protein filamin-A. However, the contributions of individual serine phosphorylation on this interaction have never been examined.

Aims: To determine the contributions of serine 253 and 258 phosphorylation on the interaction of TF with filamin-A.

Methods: Biotinylated 19-mer peptides corresponding to the C-terminus of TF, synthesised in non-phosphorylated, single-phosphorylated and double-phosphorylated forms were incubated in 96-well plates pre-coated with recombinant filamin-A (repeats 22–24) or BSA. Bound peptides were detected using streptavidin-HRP and dissociation constants (K_d) calculated.

Results: The serine 253-phosphorylated TF peptide and the double-phosphorylated TF peptide exhibited the highest binding affinities for recombinant filamin-A, while the 258-phosphorylated peptide had the lowest binding affinity. Unlabelled 253-phosphopeptide was able to compete out binding of the biotinylated 258-phosphopeptide by up to 60%, but had no significant influence on the binding of the double-phosphorylated peptide. Furthermore, a polyclonal anti-filamin-A antibody competed out binding of the phospho-serine 253 TF peptide to repeats 22–24 of filamin-A.

Conclusion: Phosphorylation at serine 253 alone is sufficient for high affinity binding of TF to filamin-A, and remains unaltered by additional phosphorylation of serine 258. This data demonstrates that the phosphorylation of serine 253 results in the formation of a filamin-A docking site within the cytoplasmic domain of TF. Individual phosphorylation of TF at serine residues 253 and 258 therefore acts as a molecular switch, enhancing and reducing the interaction between TF and filamin-A depending on the phosphorylation state of TF.

Disclosure of Interest: None declared.

PO554-MON

The interplay between tissue factor and alternatively spliced tissue factor and its effect on thrombosis and hemostasis

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Background: Plasma analysis of thrombotic patients shows elevated concentrations of tissue factor-positive microparticles (TF⁺ MP). These MPs are small vesicles, shed via blebbing of the cell membrane. The presence of tissue factor (TF), the initiator of coagulation, on these MPs causes a procoagulant state, and therefore an increased risk for thrombosis. An alternatively spliced isoform of TF (asTF), expressed in CD14⁺ macrophages and several organs like heart, lung and pancreas, is not membrane-bound but soluble.

Aims: The interplay between TF and asTF has not been studied, nor the effects of asTF on thrombosis and hemostasis. Therefore we intended to investigate the effects of asTF on shedding of TF⁺ MPs from endothelial cells and on its coagulant activity.

Methods: Endothelial cells were adenovirally transduced to express TF with and without asTF. After a 3 h culture period in serum free media, MPs were collected via centrifugation at 20000 g for 1 h. TF activity was measured on both cells and MPs using a FXa chromogenic substrate assay.

Results: The presence of asTF induced a 2-fold reduction in TF activity. Increasing FVIIa concentrations did not result in elevated FXa conversion ruling out that asTF sequesters FVIIa. Rather, western blot analysis showed that TF antigen levels went down by two-fold after co-expression of asTF. We observed less TF expression on non-raft membrane fractions of the cell, whereas lipid raft fractions exposed comparable TF antigen in the presence and absence of asTF. Inhibiting protein degradation with the proteasome inhibitor MG132 resulted in increased asTF levels, but did not result in restored TF protein expression.

Conclusion: These data indicate that TF/asTF co-expression decreases coagulant activity of TF both on cells and TF⁺ MPs. This is due to decreased TF antigen levels on the cell surface independent of proteasome-dependent TF degradation.

Disclosure of Interest: None declared.

PO555-MON

K63-linked polyubiquitination of lysine 255 within tf promotes selective TF-dephosphorylation, triggers TF-endocytosis and terminates TF activity

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Background: Restriction of cell surface activity and release of tissue factor (TF) is critical for prevention of excessive coagulation. We previously showed that the de-phosphorylation of serine 253 occurs by the action of phosphatase PP2A and leads to the termination of the release of TF within microvesicles.

Aims: To examine the role of TF ubiquitination in the regulation of TF phosphorylation, release and activity.

Methods: The plasmid for the expression of the wild type TF-tGFP was mutated to contain arginine-substitution of lysine 255 (TF_{R255}). Wild type and mutant plasmids were transfected into MDA-MB-231 cell line and HCAEC primary endothelial cells which were then permitted to express the protein. TF was immunoprecipitated from PAR2-AP and FVIIa activated cells, using tGFP and control IgG magnetic beads and analysed by western blot. Analysis was carried out

to detect tGFP, TF, polyubiquitin, TF-phosphoserine-258 and TF-phosphoserine-253 (phosphorylated PKC substrate). In addition microvesicles were isolated from the cells and the level of TF-tGFP release assessed. Finally, cellular localisation of TF was analysed by confocal microscopy.

Results: Analysis of the wild type and mutant TF-tGFP showed similar levels of tGFP, TF and phosphorylation at serine 258. However, the mutant form of TF (TF_{R25S}) lacked any ubiquitination and remained phosphorylated at serine 253 at a time-point when the wild-type TF was de-phosphorylated. In agreement with our other data, arginine-substitution of residue 255 resulted in extending the release of TF-tGFP as microvesicles and also prolonged the surface exposure of the protein.

Conclusion: Polyubiquitination of lysine 255 occurs to contain the activity and release of TF, likely by forming a platform for phosphatases to bind and de-phosphorylate serine 253 and terminating TF release. Additionally, the K63-polyubiquitination acts to form a site for interaction with proteins which mediate endocytosis.

Disclosure of Interest: None declared.

PO556-MON

Characterisation of TF ubiquitination: involvement of MDM2 and the UBE2D family of E2 enzymes

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Background: Ubiquitination of membrane receptors induces endocytosis, degradation, or alternatively change in signalling function of the receptor proteins. Characterisation of the ubiquitin-linkage gives clues to these outcomes, while identification of the ubiquitination enzymes gives clues to the underlying cellular mechanisms.

Aims: To characterise the type of ubiquitin-linkage formed and to identify the enzymes responsible for the ubiquitination of TF.

Methods: TF was immunoprecipitated from MDA-MB-231 or HCAEC primary endothelial cell lysate and analysed by western blot using antibodies discriminating against K48-linked, K63-linked ubiquitin and a non-discriminating antibody. TF was then subjected to digestion with nine de-ubiquitination enzymes (DUB). In addition, combinations of E1 and E2 enzymes were used with fraction S100 or recombinant Mdm2 protein to identify enzymes capable of ubiquitinating TF. The assay was performed using biotinylated 19-mer peptides of the C-terminus of TF (TFc), synthesised in non-phosphorylated, single-phosphorylated and double-phosphorylated forms as substrates. The peptides were then captured and ubiquitination determined using an HRP-conjugated anti-ubiquitin antibody.

Results: Immunoprecipitated cellular TF was detected by the K63-polyubiquitin and non-discriminating ubiquitin antibody but not by K48-polyubiquitin. In addition, only digestions with K63-specific DUBs (AMSH, TRABID and USP2) were capable of releasing ubiquitin. Of the six E2 enzymes, only members of the Ube2d family were able to ubiquitinate the TFc peptide. Moreover, significant ubiquitination was only observed when using double-phosphorylated TFc peptide. Finally, Mdm2 was identified as a potential E3 enzyme.

Conclusion: We have characterised the ubiquitination pattern of TF which appears to involve K63-linked ubiquitin. This reaction involves the participation of the Ube2d-family of E2 ubiquitin ligases and involves Mdm2 and requires double-phosphorylation of TF.

Disclosure of Interest: None declared.

PO557-MON

High level of tissue factor induces cell apoptosis in endothelial cells, mediated through activation of P38 and P53

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Background: The ability of TF to initiate endothelial cell proliferation is well known. In contrast, at high concentrations of TF, this is accompanied with the arrest of the progression through the cell cycle, leading to apoptosis.

Aims: In this study, TF-mediated mechanisms of induction of apoptosis were explored.

Methods: Endothelial cells were transfected to express wild-type TF. Additionally, cells were transfected to express Ala253-substituted TF to prevent TF release. Alternatively, cells were pre-incubated with TF-rich and TF-poor microvesicles or microvesicles derived from patients with cardiovascular complications or normal individuals. Cell apoptosis and the expression of p53 and Bax were measured following activation of cells with PAR2-agonist peptide.

Results: Greatest levels of cellular apoptosis was observed in cells expressing Ala253-substituted TF, or cells pre-incubated with TF-rich or patient microvesicles. The level of p53 protein, p53-phosphorylation at ser33, p53 nuclear localisation and transcriptional activity, but not p53 mRNA, were increased in cells expressing wild-type and Ala253-substituted TF, or in cells pre-incubated with TF-rich or patient microvesicles. However, the expression of Bax protein was only increased in cells pre-incubated with TF-rich or patient microvesicles, and in cells expressing Ala253-substituted TF. Inhibition of the transcriptional activity of p53 using pifithrin- α suppressed the expression of Bax. Finally, siRNA-mediated suppression of p38 α , or inhibition using SB202190 significantly reduced p53 protein levels, p53 nuclear localisation and transcriptional activity, suppressed Bax expression and prevented cellular apoptosis.

Conclusion: In conclusion, accumulation of TF within endothelial cells, or sequestered from their surroundings can induce cellular apoptosis through mechanisms mediated by p38, and involves the stabilisation of p53.

Disclosure of Interest: None declared.

PO558-MON

The potential of cancer cell lines to release tissue factor-containing microvesicles correlates with tissue factor and PAR2 mRNA levels but not tissue factor protein or surface activity

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Background: Cells may release microvesicles upon activation and depending on the stimuli, these microvesicles may harbour TF. The exposure of cancer cells to proteases may activate PAR2 and prompt dormant TF-bearing tumour cells to release large quantities of TF-bearing microvesicles.

Aims: In this *in vitro* study, we have examined the potential of cells to release TF as microvesicles in different cells lines and assessed the correlation to TF and PAR2 expression.

Methods: Microvesicle release was induced by PAR2 activation in seventeen cancer cell lines and the microvesicles were isolated. Microvesicle-associated TF activity was then measured. Furthermore, TF mRNA expression, cellular TF protein and cell-surface TF activities were quantified in all cell lines. Finally, the relative expression of PAR2 mRNA and cellular protein were analysed. Any correlations between these parameters were examined by determining the Pearson's correlation coefficients.

Results: TF release as microvesicles peaked between 30–60 min post-activation in the majority of cell lines tested. The magnitude of the maximal TF release positively correlated with TF mRNA ($c = 0.717$; $P < 0.001$) and PAR2 mRNA ($c = 0.770$; $P < 0.001$) expressions while the percentage increase correlated with PAR2 mRNA ($c = 0.601$; $P = 0.011$) and protein ($c = 0.714$; $P < 0.001$). In contrast, TF release in resting cells did not significantly correlate with any of the parameters examined.

Conclusion: In conclusion, this study suggests that while PAR2 may maintain the level of TF expression, the ability of cells to release TF as cell-derived microvesicles may be upregulated rapidly and significantly following the activation of PAR2. Moreover, quantification of the levels of TF and PAR2 mRNA and possibly PAR2 protein, may prove to be a means of determining the potential of cancer cells to release TF-containing microvesicles and constitute a more accurate predictor of risk of thrombosis *in vivo*.

Disclosure of Interest: None declared.

PO559-MON

Tissue factor is induced by interleukin-33 in human endothelial cells: a new link between coagulation and inflammation

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Background: Tissue factor (TF) is the primary trigger of coagulation. Elevated levels of TF are found in atherosclerotic plaques, and TF leads to thrombus formation, when released upon plaque rupture. Interleukin (IL)-33 induces angiogenesis and inflammatory activation of endothelial cells.

Aims: Here we investigated the impact of IL-33 on TF in human endothelial cells, as a new possible link between inflammation and coagulation.

Methods: Human umbilical vein endothelial cells (HUVEC) and human coronary artery endothelial cells (HCAEC) were treated with IL-33 alone, or together with ST2 receptor antibody (ST2AB), or IL-1 receptor antibody (IL1RA), or infected with an adenovirus overexpressing IkBa (AdV-IkBa). Effects of IL-33 on TF expression and activity were measured by RT-PCR, ELISA, FXa generation and flow cytometry. Rotational thromboelastometry was used to determine the effect of IL-33 activated EC on clotting time of whole blood and plasma from healthy volunteers. Human carotid atherosclerotic plaques were stained for IL-33 and TF using fluorescence immunohistochemistry. Total RNA was isolated from same plaques.

Results: IL-33 significantly induced TF mRNA and protein expression in HUVEC and HCAEC. IL-33 also increased cell surface TF protein expression and activity levels as well as TF activity in EC-derived microparticles. IL-33 activated EC reduced coagulation time of whole blood and plasma. ST2AB and AdV-IkBa inhibited IL-33-induced TF mRNA expression in HUVEC. IL-1RA had no effect on IL-33-induced TF mRNA expression. In human carotid atherosclerotic plaques ($n = 50$), TF mRNA positively correlated with IL-33 mRNA expression ($r = 0.691$, $P < 0.001$). IL-33 and TF protein were detected in endothelial cells of atherosclerotic plaques and co-localized with FXIIIa at the site of clot formation within microvessels in symptomatic plaques.

Conclusion: Via induction of TF in endothelial cells IL-33 could enhance their thrombotic capacity and thereby might impact on thrombus formation in the setting of atherosclerosis.

Disclosure of Interest: None declared.

PO560-MON

Soluble tissue factor improves detection of thrombin generation responses to wide range of factor VIIa doses

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Background: Coagulation Factor VIIa (FVIIa) is used for treatment of bleeding in hemophilia patients with inhibitors. Clinical responses to FVIIa may vary among patients, suggesting that laboratory-guided dose-optimization may improve treatment outcome. Although Thrombin Generation Test (TGT) is widely used for clinical evaluations of FVIIa, its utility remains controversial. For example, TGT response is nearly unchanged for several hours after FVIIa administration, in sharp contrast with both FVIIa pharmacokinetics and current recommendation to repeat dosing every 2 h.

Aims: We tested the hypothesis that engineered tissue factor (TF) variants with low FVIIa affinity can remove saturation of TGT responses, extending assay sensitivity to a wide therapeutic range of FVIIa concentrations.

Methods: Soluble TF variants with reduced affinity to FVIIa were compared with common commercial TF or procoagulant lipid (PL) reagents for TGT analysis of hemophilia A and Factor VII-deficient plasma spiked with two marketed recombinant FVIIa products or GLA-domainless FVIIa. Mathematical model was used to predict pharmacokinetics-pharmacodynamics (PK/PD) *ex vivo*.

Results: In agreement with previous studies, flat dose responses were observed in the pharmacological FVIIa range for any full length TF reagent, and no responses below 40 $\mu\text{g kg}^{-1}$ dose were seen without TF. In the presence of optimal soluble TF concentration, thrombin generation was linearly proportional to FVIIa concentrations corresponding to 2–400 $\mu\text{g kg}^{-1}$ doses. Simulated PK/PD experiments predicted assay ability to detect changes in TGT up to 15 h after single 270 $\mu\text{g kg}^{-1}$ administration.

Conclusion: Low binding TF-based TGT may offer improved assessment of FVIIa-induced thrombin generation potential *ex vivo*. Further evaluation under clinical setting conditions is needed to establish the utility for PK/PD evaluation of FVIIa therapies.

Disclosure of Interest: A. Shibeko: None Declared, A. Rezaie: None Declared, T. Lee: None Declared, M. Ovanesov Employee of: This paper is an informal communication and represents authors' best judgment. These comments do not bind or obligate FDA.

PO561-MON

Naturally occurring neisseria meningitidis with mutated lipid A moiety is a weaker inducer of tissue factor expression and activity in human monocytes and monocyte-derived microvesicles than the wildtype meningococci

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Background: Tissue factor (TF) is the main initiator of coagulation. During meningococcal infections, lipopolysaccharides (LPS) in the bacterial outer membrane may induce TF in monocytes and microvesicles (MVs). The LPS molecules of wildtype (wt) *Neisseria meningitidis* (*Nm*) have a lipid A moiety with six fatty acyl chains. However, other naturally occurring *Nm*-strains with only five fatty acyl chains in the lipid A moiety (lpxL1 mutants) have been documented in patients with meningococcal disease. These patients have less systemic inflammation, higher thrombocyte counts and less frequently rash than patients infected with wt meningococci.

Aims: To explore how the *Nm* lpxL1 mutant affects TF-expression and TF-activity of primary human monocytes and monocyte-derived MVs.

Methods: Monocytes from three donors were grown $\pm$ heat-inactivated wt or lpxL1 meningococci (10^4 mL⁻¹). Cells were pelleted (300 g, 10 min) and subjected to RNA-, flow- and calibrated automated thrombogram (CAT) analysis. MVs were isolated from the cell supernatant (17,000 g, 30 min), and analyzed with CAT.

Results: RNA (2 h): TF RNA was upregulated 7–30 fold (wt) and 1–4 fold (lpxL1), both compared with unstimulated samples.

FLOW (4 h): Percentage TF-positive monocytes were 46–49% (wt), 14–27% (lpxL1) and 10–17% (unstimulated).

CAT (4 h): Cells incubated with wt induced prominent thrombin generation (lagtime (LT) 3.5–4.0 min, velocity index (VI) 4.9–6.8 nm min⁻¹) compared with cells incubated with the lpxL1 mutant (LT: 6.2–7.2 min, VI: 1.1–1.7 nm min⁻¹) and unstimulated cells (LT: 7.3–10.9 min, VI: 0.2–1.1 nm min⁻¹). The trend was similar for MVs: wt (LT: 2.7–3.3 min, VI: 14.4–22.3 nm min⁻¹), lpxL1 (LT: 8.3–10.5 min, VI: index 0.7–1.3 nm min⁻¹) and unstimulated (LT: 8.7–22.3 min, VI 0.2–0.7 nm min⁻¹). All thrombin generation curves were markedly reduced when the cells and MVs were incubated with anti-TF antibodies.

Conclusion: Compared with wt *Nm*, the lpxL1 mutant induces lower TF-expression and TF-activity on human monocytes, and also lower MV-associated TF-activity.

Disclosure of Interest: None declared.

PO562-MON

Do elevated coagulation factor levels affect the tissue factor-threshold in thrombin generation?

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Background: High procoagulant or low anticoagulant factor levels are known to associate with an increased risk for venous thrombosis (VT). However, the exact molecular mechanism by which these altered factor levels modulate the risk is incompletely understood.

Aims: Here we hypothesize that elevated factor levels affect the pro- and anticoagulant balance in coagulation such that even minute amounts of tissue factor (TF) will initiate a full-blown coagulation response, thereby contributing to the VT risk.

Methods: To test this so-called TF-threshold hypothesis, we monitored thrombin generation initiated by very low TF concentrations (0–500fM) in factor XII-deficient plasma, to avoid of any contact pathway-mediated thrombin formation.

Results: In this system, thrombin generation occurred ≥ 15 fM TF. Increasing the factor VIII (FVIII; Aaact levels (150%; 200%)) resulted in enhanced thrombin formation, characterized by an increase in the thrombin peak and endogenous thrombin potential (ETP) at the higher TF concentrations (150–500fM), while the lag time and time-to-peak were not affected. However, increasing the plasma FVIII concentration up to two-fold did not alter the TF-threshold substantially. Similar findings were obtained upon additions of prothrombin (plasma levels of 150% or 200%). Conversely, thrombin generation was dramatically impaired with 150% or 200% antithrombin (AT) present, demonstrated by a reduced peak thrombin and ETP (2- or 4-fold). Consistent with this, we observed a shift in the TF-threshold to ~ 150 fM (150% AT) and 500 fM (200% AT).

Conclusion: In summary, although elevated plasma levels of FVIII or prothrombin affect parameters of thrombin generation, they do not reinforce the TF-dependent initiation of coagulation. As such, our data do not support a mediating role for the TF-threshold in the association between high procoagulant factor levels and the risk for VT. High AT concentrations do cause an upward TF-threshold shift, which may be associated with a protective effect on risk for VT.

Disclosure of Interest: None declared.

PO563-MON

Tissue factor/factor VIIA signaling promotes cytokine-induced beta cell death and impairs glucose stimulated insulin secretion from human pancreatic islets

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Background: Patients diagnosed with type 1- or type 2-diabetes show elevated levels of coagulation factor VIIa (FVIIa) and its receptor Tissue factor (TF) in their blood stream that may affect the fate of the beta cells.

Aims: We aimed to study the effect of TF/FVIIa signaling on cytokine-induced beta cell death and islet function *in vitro*.

Methods: Human pancreatic islets and MIN-6 beta cells were used to study TF mRNA and protein expression using real time PCR and immunoblotting. Cell surface TF was determined using flow cytometry. TF/FVIIa effects on cytokine-induced beta cell death were studied in MIN-6 cells using a cell death ELISA. Effects of TF/FVIIa on the phosphorylation of p38, ERK and JNK were investigated using immunoblotting. Glucose stimulated insulin secretion (GSIS) from human pancreatic islets was measured with an insulin ELISA.

Results: A combination of the pro-inflammatory cytokines interleukin-1beta, tumour necrosis factor-alpha and interferon-gamma, induced TF expression in human pancreatic islets and in beta cells. TF/FVIIa did not affect basal beta cell death but, independently of downstream coagulation activity, augmented beta cell death in response to cytokine treatment. The effect of TF/FVIIa on cytokine-induced beta cell death was found to be dependent on the stress kinase JNK, since FVIIa addition potentiated cytokine-induced JNK activation and JNK inhibition abolished the effect of TF/FVIIa on cytokine-induced beta cell death. Moreover, TF/FVIIa signaling resulted in inhibition of GSIS from isolated human pancreatic islets.

Conclusion: These results indicate that TF/FVIIa signalling has a negative effect on beta cell function and promotes beta cell death in response to cytokines.

Disclosure of Interest: None declared.

PO564-MON

Tissue factor activation in pregnancy complications

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Background: TF is a specific and high-affinity receptor for factor VII/VIIa and acts as a cofactor for Factor VIIa. Exposure of activated TF in coagulation system triggers the physiological blood coagulation and thrombosis in a number of thrombotic diseases

Aims: Analysis of changes in expression of tissue factor inhibitor, including with regard to their impact on the initiation of activation of coagulation changes that can lead to pregnancy complications in healthy pregnant women compared with pregnant women treated for chronic hypertension, diabetes mellitus and preeclampsia.

Methods: We have proposed a model monitor activation of the coagulation system in preeclampsia and other pregnancy complications with TF expression on monocytes by flow cytometry and simultaneously fixing the TF-induced thrombin generation in plasma. To determine expression of tissue factor (CD142) on monocytes, we used the method of multicolor flow cytometry using anti CD45 PerCP, clone MEM-28, anti CD14 APC clone MEM-15, CD16b FITC clone MEM-154, anti CD142 PE and the appropriate isotope control. Peripheral blood of 198 prospectively monitored pregnant patient with signed informed consent have been analyzed. Test were done at the beginning of pregnancy – up to end the first trimester. The second collection was done in the period 24 to 28 week, the third sample after the 36th – 40th weeks.

Results: We have confirmed higher expression of CD142 and CD 14 (TF) and monocytes in patients with developed preeclampsia compared to healthy controls ($P < 0.01$).

Conclusion: The detection of CD142 expression by flow cytometry seems to be an effective method of monitoring the activation of the coagulation system and methodology developed appears to be very promising for further uncovering the mechanisms of preeclampsia and conditions associated with it.

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Disclosure of Interest: None declared.

PO565-MON

Activated platelets regulate monocyte tfpi gene expression through the nuclear transcription factor PPAR γ

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Background: Tissue Factor Pathway Inhibitor (TFPI) is primarily expressed on endothelial cells and macrophages. We have previously shown that *tfpi* mRNA is also induced in monocytes by GPVI-activated platelets.

Aims: To investigate the mechanism by which platelets regulate monocyte *tfpi* expression.

Methods: Monocytes were stimulated by CRP-XL-activated platelets and isolated by immune-magnetic beads. Relative expression of *tfpi* was measured using RT-qPCR. Oxylipins were extracted from the releasate of activated platelets using a hexane-based method for analysis by LC-MS/MS. Activation of PPAR γ was measured by luciferase expression in 293T cells relative to β -galactosidase.

Results: GPVI-activated platelets induced monocyte *tfpi* expression in a time dependent manner, reaching maximum at 6 h ($P = 0.0021$; $n = 4$). Preventing platelet monocyte aggregates with the CD62P-blocking antibody 9E1 gave no change in expression indicating material released from platelets induces *tfpi* expression. When the releasate from activated platelets was separated into protein or oxylipin fractions and incubated with monocytes, oxylipins were identified as responsible for the induction of *tfpi* expression ($P = 0.0009$; $n = 9$). LC-MS/MS analysis identified 16 oxylipins of which, 12-/15-HETE, 9-/13-HODE and 9-oxoODE are known PPAR γ agonists. Blocking formation of these oxylipins with aspirin and esculetin resulted in reduced *tfpi* expression ($P = 0.0465$ and 0.0153 respectively; $n = 3$) and dual inhibition was additive ($P = 0.0024$; $n = 3$). Conversely *tfpi* expression was induced by the known PPAR γ agonists rosiglitazone ($P = 0.032$) and 15-PGJ2 (0.0087 ; $n = 3$). PPAR γ activation by 12-/15-HETE was confirmed in a transactivation assay ($P = 0.0285$ and 0.0006 respectively; $n = 6$).

Conclusion: These results suggest activated platelets induce expression of *tfpi* in monocytes through the release of AA-derived PPAR γ agonists. This effect could be a therapeutic target in the prevention of coronary artery disease.

Disclosure of Interest: None declared.

PO566-MON

Tissue factor and tissue factor pathway inhibitor assessment in early and late severe preeclampsia

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Background: Preeclampsia (PE) is a multi-system disorder of human pregnancy characterized by hypertension (blood pressure $\geq 140 \times 90$ mmHg) and proteinuria (≥ 0.3 g protein in 24-h specimen) occurring after the 20th week of pregnancy. According to gestational age (GA) at the onset of disease, PE has been classified as early (GA < 34 weeks) and late (GA ≥ 34 weeks). PE is associated with increased hypercoagulable state compared to normotensive pregnant. Tissue factor (TF) is the main physiological initiator of blood coagulation. *In vivo*, TF activity is regulated by a specific inhibitor known as Tissue factor pathway inhibitor (TFPI).

Aims: To assess FT (pg mL⁻¹) and TFPI (ng mL⁻¹) and TF/TFPI ratio plasma levels in early and late severe PE and normotensive pregnant.

Methods: This study was approved by local Ethical Committee and consent was obtained in all cases. It included four groups: G1: early severe PE women ($n = 30$); G2: late severe PE women ($n = 25$); G3: early normotensive pregnant ($n = 34$) and G4: late normotensive pregnant ($n = 14$).

TF and TFPI plasma levels were determined by ELISA.

Results: The mean $\pm$ standard deviation for TF were: G1 = 324.9 ± 120.2 ; G2 = 284.9 ± 112.6 ; G3 = 270.9 ± 116.3 and G4 = 260.9 ± 29.2 and no significant difference was found among these groups. For TFPI, median (Interquartile range) were G1 = 104.6 (53.1); G2 = 60.2 (51.5); G3 = 62.2 (24.5) and G4 = 65.0 (50.7). TFPI levels were higher in G1 and lower in G2 comparing to G3. No difference was found comparing G1, G2 and G3 to G4. The median (Interquartile range) for TF/TFPI ratio were: G1 = 2.64 (2.89); G2 = 3.80 (3.88); G3 = 3.71 (3.57) and G4 = 4.19 (5.31) and was lower in G1 comparing to G3 and G4.

Conclusion: Although TF levels was not increased in early severe PE comparing to normotensive pregnant before 34 weeks of gestation, higher levels of TFPI were found. It can be speculate an increase of TF

in uterine environment to justify the high circulate TFPI in early severe PE. A decrease of TF/TFPI ratio in early severe PE supports the hypothesis.

Disclosure of Interest: None declared.

Transfusion I

PO567-MON

Levonorgestrel-releasing intra-uterine device use in adolescent females with heavy menstrual bleeding and bleeding disorders: single institution review

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Background: There is limited data on the use of Levonorgestrel-releasing intra-uterine device (LNGIUD) in adolescents with HMB due to bleeding disorders (BD).

Aims: We hypothesized that LNGIUD would be efficacious for post-menarchal adolescents with HMB and BD.

Methods: An IRB approved retrospective chart review was undertaken. BD diagnoses, menstrual cycle details, laboratory values and HMB treatment details at baseline and after LNGIUD placement were assessed. Pictorial Blood Assessment Chart scores > 100, HMB requiring pad/tampon change every 1–2 h, passage of clots, or cycles lasting for ≥ 7 days qualified as HMB. Descriptive statistics and t-tests were utilized.

Results: Thirteen patients met inclusion criteria. The mean age of diagnosis of HMB was 14.08 ± 1.75 years. BD diagnoses included low von Willebrand (VW) activity (5), type I VW disease (VWD) (5), type IIM VWD (1), low VW activity and factor 7 deficiency (1), acquired VW activity and factor 7 deficiency (1). Prior to LNGIUD, hormonal therapy and hemostatic therapy (antifibrinolytic agents/DDAVP) administered alone, yielded poor control of HMB. IUD was placed with periprocedure hemostatic therapy with no resultant HMB or other complications. All patients reported significant improvement in HMB after LNGIUD placement with mean time to improvement of 97.0 ± 71.3 days, and 60% achieved amenorrhea within the timeframe. Mean hemoglobin level pre LNGIUD was 11 ± 3.5 mg dL⁻¹ and post LNGIUD was 13.4 ± 0.9 mg dL⁻¹. There was a significant rise in Hb post LNGIUD ($P = 0.0264$). Use of supplemental hormonal or hemostatic agents was low after LNGIUD (4). None required IUD removal; 1 spontaneously expelled the IUD with subsequent replacement.

Conclusion: Our study results indicate that the LNG IUD can be an effective therapeutic option in post-menarchal adolescent girls with HMB due to BD. In addition, the LNGIUD is associated with minimal complications, high compliance rates in adolescents, and improvement in HMB and anemia.

Disclosure of Interest: None declared.

PO568-MON

A new alloantigen (CAB4) carried by GPIX identified in a context of severe neonatal alloimmune thrombocytopenia

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Background: After 3 miscarriages, at 1 year of interval a 39-year-old woman gave birth to 2 thrombocytopenic infants (6 and 30 G/L) with

intracranial haemorrhages. Thrombocytopenias were corrected with transfusion of apheresis platelets but if the outcome was favourable for the 1st infant, the 2nd died 10 days after she was delivered at 33 WA + 6 days by caesarean.

Aims: The study aimed at characterizing the origin of these thrombocytopenias.

Methods: MAIPA and flow cytometry techniques were used for serological studies. Genotyping of human platelet antigens (HPA) were done using Beadchips and PCR-SSP techniques. Gene variant was identified by direct sequencing of PCR products and the mutant glycoprotein (GP) was expressed in transiently transfected HEK293 cells.

Results: The 1st neonate showed no materno-foetal incompatibilities for HPA 1 to 6, and no maternal antibody to GPIaIIa, IIbIIIa or IbIX were found. For the 2nd infant, the maternal serum faintly reacted with GPIbIX from paternal platelets but not with maternal or panel platelets. Except for HPA-1b, no materno-foetal incompatibility was identified for other HPA antigens. A new alloantigen carried by GPIbIX was suspected. A paternal c.368C>T variation leading to a p.Pro123Leu substitution was identified in GPIX. The 2nd neonate was heterozygous c.368C/T and not compatible with the mother homozygous c.368C. The 1st infant's genotype remains to be studied. The p.Leu123 form of GPIbIX was normally expressed at the surface of transfected HEK293 cells and was responsible for the maternal anti-GPIbIX serum reactivity. The allelic frequency of the GPIX c.368T allele is currently under study.

Conclusion: We report here the first alloantigen carried by GPIX. It was identified in a context of severe life-threatening thrombocytopenia associated with materno-foetal incompatibility in the HPA-1 system but without detected antibody to HPA-1b. Indeed, the role of the anti-GPIbIX alloantibody explaining the severity of both thrombocytopenias requires further studies.

Disclosure of Interest: None declared.

PO569-MON

Assessment of quality and clinical effectiveness of apheresis platelet concentrates treated with mirasol pathogen reduction system in plasma or in platelet additive solution SSP+

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Background: Mirasol treatment can reduce the risk of transfusion-transmitted infections, however, it also results in reduced platelet quality and increased markers of activation during storage.

Aims: We compared storage characteristics and clinical effectiveness of Mirasol-treated and non-treated apheresis platelet concentrates (PCs) stored in plasma or in additional solution SSP+.

Methods: PCs were obtained from healthy donors with their written informed consent. PCs were collected using Trima Accel apheresis system into autologous plasma or into 40% plasma/60% SSP+. Activation markers exposure was analyzed using flow cytometry before and after activation on 0, 1, 3 and 5 days of storage. The clinical effectiveness study included transfusions of PCs stored in plasma or SSP+ and treated or non-treated with Mirasol for patients with acute lymphoblastic leukemia or acute myelogenous leukemia, who had a hemorrhagic syndrome of less than 2 points on the WHO scale.

Results: A significant increase in the expression of platelet activation markers was observed during the storage period in all groups of PCs. P-selectin and phosphatidylserine expression in Mirasol-treated samples were significantly higher compared to the control ones already on the day of harvesting, and continued to grow during the storage. On the 5th day of storage, 20–50% of platelets treated with Mirasol did not respond to the activation. This result agreed well with the sig-

nificant decrease (approximately 2-fold) in the effectiveness (we compared the absolute and corrected platelets count increment at 24 h after transfusion) of transfusions of PCs treated with Mirasol. Transfusions of PCs stored in SSP+ were accompanied by a fewer inefficient transfusions and post-transfusion reactions than of PCs stored in plasma.

Conclusion: In summary, functional studies revealed that treatment with Mirasol had an impact on platelet function that was particularly profound on the 5th day of storage and led to a decrease in the effectiveness of transfusions of PCs.

Disclosure of Interest: None declared.

PO570-MON

Quantification of cell free DNA in red blood cell concentrates produced via buffy coat (B1) OR whole blood (B2) methods

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Background: Whole blood (WB) donations in many countries are processed by the buffy coat (B1) method in which leukoreduction occurs within 20 h; or B2, within 72 h. This delay in leukoreduction may result in release of DNA from cells (cfDNA), which associate with histones. Several reports have shown significant association between transfusion of fresh (≤ 2 weeks old) B2 red cells (RC) and in-hospital mortality, which may be mediated via cfDNA. cfDNA can activate coagulation and is associated with an increased risk of mortality in septic patients. Histones activate platelets and are cytotoxic to vascular endothelial cells.

Aims: To test our hypothesis that cfDNA levels are increased in B2 units (for which leukoreduction is delayed) compared to B1; and to assess if levels are impacted by duration of storage.

Methods: RCs destined for transfusion were selected with equal numbers of fresh (< 2 weeks) and older (≥ 2 weeks) RCs. Samples of these units were obtained and the cfDNA isolated. The cfDNA concentration was then quantified by spectrophotometry and PicoGreen; and for nucleosomes (cfDNA-histone complexes) via ELISA. Analysis looked at the levels of cfDNA by WB processing method and duration of RC storage.

Results: 127 RC units were tested: 73 B1, 54 B2. By spectrophotometry, fresh RCs compared to older RCs had significantly higher cfDNA (3.66 ± 1.69 vs 2.98 ± 1.49 $\mu\text{g mL}^{-1}$, $P < 0.001$) and a trend of higher cfDNA with B2 RCs compared to B1 RCs (3.56 ± 1.99 vs 3.28 ± 1.28 $\mu\text{g mL}^{-1}$, $P = 0.06$). Using PicoGreen, B2 RCs had higher cfDNA (1.08 ± 0.9 vs 0.5 ± 0.77 ng mL^{-1} , $P < 0.001$). Nucleosome ELISA confirmed the presence of DNA-histone complexes (0.17 ± 0.13 $\mu\text{g mL}^{-1}$).

Conclusion: We demonstrate higher cfDNA in fresh blood and in B2 units. These findings are consistent with the clinical observations that fresh B2 blood may be associated with increased mortality in transfused patients. Further studies are required to confirm these observations and to understand the pathobiology.

Disclosure of Interest: None declared.

PO571-MON

Responsiveness of platelets during storage studied with flow cytometry – formation of platelet subpopulations and LAMP-1 as new markers for the platelet storage lesion

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Background: Storage of platelet concentrates (PCs) results in a platelet storage lesion which may reduce the hemostatic function of transfused platelets. Quality testing of PCs commonly involves analysis of blood gases, metabolic variables and spontaneous expression of platelet activation markers. However, response towards agonists is rarely studied.

Aims: The aim of this study was to find new and potentially more sensitive flow cytometry markers to detect changes in platelet function during storage.

Methods: PCs prepared by apheresis ($n = 6$) were examined on days 1, 5, 7 and 12. Exposure of lysosomal associated membrane protein-1 (LAMP-1), P-selectin and phosphatidylserine (PS) and formation of different platelet subpopulations was assessed by flow cytometry in resting state or following stimulation with platelet agonists (collagen-related peptide (CRP), PAR1-activating peptide (AP) and PAR4-AP). The protocol was also tested in six PCs with reduced swirling.

Results: The ability to form subpopulations upon activation was significantly decreased already on day 5 for some agonist combinations, e.g. seen as a decrease in the fraction of small platelets ($25 \pm 3\%$ on day 1 and $15 \pm 3\%$ on day 5 upon activation with CRP+PAR1-AP+PAR4-AP, mean $\pm$ SEM). In PCs with reduced swirling the ability to form subpopulations was severely reduced or even abolished. Spontaneous exposure of P-selectin and PS increased with time, while spontaneous LAMP-1 exposure was unchanged. Agonist-induced exposure of PS and LAMP-1 gradually decreased with time (from $38 \pm 5\%$ and $77 \pm 3\%$, respectively, on day 1 to $11 \pm 1\%$ and $50 \pm 9\%$ on day 12), and agonist-induced LAMP-1 exposure was absent in PCs with reduced swirling.

Conclusion: The platelet activation potential measured as agonist-induced expression of LAMP-1 and fragmentation into platelet subpopulations are potential sensitive markers that could be beneficial to include in evaluation of the platelet storage lesion.

Disclosure of Interest: None declared.

PO572-MON

Preserved ultrastructure and biochemical characteristics of platelet components prepared with amotosalen and UVA for pathogen inactivation

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Background: Although safe and efficient, platelet concentrates (PCT-PCs) treated with the Intercept[®] pathogen inactivation method (amotosalen and UVA) exhibit reduced platelet recovery after transfusion as compared to conventional untreated control (C) PCs. Platelets from PCT-PCs and from C-PCs display however similar platelet reactivity and proteomic characteristics evaluated by 2D-DIGE analysis during 6.5 days of storage (Hechler, Transfusion 2013).

Aims: To further examine the selective impact of the Intercept[®] method on the integrity and biochemical characteristics of platelets from PCT-PCs compared to C-PCs.

Methods: Platelets from PCT-PCs or from C-PCs were isolated on day (D) 1.5 and D6.5 of storage, washed and resuspended in Tyrode's buffer and compared in terms of their ultrastructural morphology by transmission electron microscopy, expression of the 'don't eat me' signal CD47 by flow cytometry and proteomic profile using the iTRAQ (isobaric tags for relative and absolute quantitation) approach.

Results: At D1.5, 85% of C-platelets and 93% of PCT-platelets displayed disk shape. At D6.5, 63% of both C- and PCT-platelets still retained their disk shape, while the remaining 37% of platelets displayed similar signs of activation. Surface expression of CD47 remained stable during storage and no differences were detected between C- and PCT-platelets. Using the iTRAQ approach to have a global look at platelet protein changes, a total number of 1314 unique proteins were identified. Relative expression data indicated that only 3 proteins were differently modified between C- and PCT-platelets by D1.5 and 10 proteins by D6.5 by more than 1.5-fold (P -value<0.05). These proteins are linked to the cytoskeleton, oxidative stress and platelet metabolism.

Conclusion: The Intercept® pathogen inactivation method has no major impact on platelet ultrastructure, expression of CD47 and platelet protein profile during 6.5 days of storage.

Disclosure of Interest: None declared.

PO573-MON

Platelet transfusions at the clinic of chemotherapy: a hospital-based cohort study

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Background: Thrombocytopenia is a significant complication of cancer therapy and has been known for decades. Platelet transfusion is the most accepted method to treat this complication and, indeed, one of the main indications of platelet transfusion is myelosuppressive chemotherapy. Unfortunately, the data in this area is quite limited, and the knowledge gap is even larger from the countries with limited resources.

Aims: The aims of this study were to assess the platelet transfusion rates in patients with cancer receiving chemotherapy and to describe the complications related to those transfusions.

Methods: A hospital-based cohort study was conducted at the Clinic of Chemotherapy of Muratsan Hospital Complex of Yerevan State Medical University, involving all cancer patients from Dec.2008 to Dec.2014. Demographic, clinical, laboratory and transfusion data was collected from the patients' database and from the hospital transfusion registry and a subsequent analysis was performed.

Results: Of 610 patients (0–78 years old) with cancer (both solid tumors and hematologic malignancies) receiving chemotherapy, 51 (8.4%) patients received in total 207 platelet transfusions. Overall, 404 platelet products were spent, of those, 36 (6.4%) were aphaeresis platelets and the other 368 (95.6%) were platelet concentrates. None of platelet products were irradiated, since it is not available in our country. Transfusion associated reactions were very rare and only 5 (2.4%) mild reactions were observed, of which 3 patients had allergic rash (one of them twice) and another 1 had febrile non-hemolytic transfusion reaction.

Conclusion: In this study we assessed the platelet transfusion rates and transfusion associated reactions in cancer patients receiving chemotherapy. However, some studies showed higher incidence of platelet transfusion related reactions, in our patient cohort those complications were rare. It may be explained by the lower number of transfused platelet units per patient in most of the cases, due to limited availability.

Disclosure of Interest: None declared.

PO575-MON

HPA beadchip genotyping study: comparison between DNA extracted from blood samples and buccal SWABS

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Background: The HPA Beadchip genotyping kit (CE-IVD BioArray Solutions, Immucor, Warren, NJ) has been introduced in the Platelet Immunology Department of the National Institute of Blood Transfusion in 2010. This technique allows to test for 11 human platelet antigen (HPA) groups simultaneously in a single platform; total time of assay is only 6 h, including the DNA extraction procedure.

Aims: The aim of the study was to compare platelet genotypes using DNA extracted from blood samples and buccal swabs in patients suffering severe thrombocytopenia, presenting with refractoriness after platelet transfusion or being pre-term neonates. Indeed, the current protocol for genotyping requires a range of DNA quantity between 80 to 640 ng at a concentration ranging from 10 to 80 ng μL^{-1} .

Methods: This study was carried out in 2013 and 2014 and concerned a total of 112 samples. Two CE-marked *in vitro* diagnostics (IVD) DNA extraction techniques were implemented: i) the automated extraction on MagNA PURE Compact with the MagNA PURE Compact Nucleic Acid Isolation Kit I (Roche Diagnostics, Gmbh); ii) and the manual QIAamp DSP DNA Blood Mini kit (Qiagen, Gmbh). The automated extraction was only used for blood samples while buccal swabs were extracted with either technique.

Results: In the 112 samples tested, 103 HPA genotyping results were 100% concordant even if the DNA concentration in buccal swabs is lower than the recommended protocol (≥ 0.5 ng μL^{-1}). 9 HPA genotyping buccal swabs results were non-interpretable; different hypotheses have been proposed to explain these results, such as DNA degradation, low DNA concentration, punctual misreading of the chip. ... These 9 samples are currently under study.

Conclusion: The HPA Beadchip genotyping kit allows a suitable genotyping of platelets antigens even at low DNA concentration. These first results indicate some interest to genotype on buccal swabs, an easy and non-invasive collecting method which could be introduced in the future in routine screening.

Disclosure of Interest: None declared.

PO576-MON

Prothrombin activation in packed red blood cell units over their storage lifetime

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Background: In *in vitro* models of blood coagulation, red blood cells (RBCs) appear to be sites of significant thrombin generation, suggesting their potential to participate in normal hemorrhage control at injury sites. In traumatic injuries with severe blood loss, packed RBC preparations (pRBCs) are transfused to improve oxygen delivery.

Aims: To examine the capacity of RBCs in pRBC transfusion units to support prothrombinase assembly and function over the 42 day shelf-life of the product.

Methods: Five units of leukocyte reduced pRBCs (A, Rh+, CPD-AS-1) were obtained from a local blood bank and each sampled at 7, 14, 21, 28, 35 and 42 days. Cell number, pH, and % lysed cells were measured, and an aliquot of cells processed to provide a washed cell fraction and a microparticle fraction. Prothrombinase assays were conducted on unwashed (total) and washed RBCs as well as volumetric

cally equivalent amounts of microparticles. Phosphatidylserine expression on washed RBCs was quantified by flow cytometry using both FITC-labeled bovine lactadherin and FITC-labeled FXa (2 nM) with 20 nM FVa. Data are expressed as mean $\pm$ SD.

Results: Between day 7 and 42 continuous changes in pH (6.75 ± 0.04 to 6.5 ± 0.08), % cell lysis (0.024 ± 0.22 to 1.33 ± 0.47) and total prothrombinase activity (~ 3.5 -fold increase) were observed. At day 7, $\sim 80\%$ of the total prothrombinase activity was associated with the washed RBC fraction and 20% with the microparticle fraction. At day 42, prothrombinase activity by the microparticle fraction had increased ~ 8 -fold, accounting for $\sim 2/3$ of the total; the level of activity of the washed RBC fraction remained stable. Consistent with this, flow cytometry showed no trend in the % of washed RBCs binding bovine lactadherin or FVa dependent FXa binding.

Conclusion: During the 42 day operational lifetime of pRBC units, increasing capacity to support prothrombinase is associated with the microparticle fraction while intact RBCs appear stable.

Disclosure of Interest: T. Orfeo: None Declared, H. Keith: None Declared, E. Lavoie: None Declared, B. Bouchard: None Declared, M. Gissel: None Declared, M. Fung: None Declared, K. Mann Shareholder of: Haematologic Technologies, Consultant for: Alnylam, Baxter, Bayer, CSL Behring, Diagnostica Stago.

PO577-MON

Exploring the challenges of effective factor distribution and management in the province of Saskatchewan

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Background: The Saskatchewan Bleeding Disorders program serves the Canadian province of Saskatchewan (SK) which is 651,900 km² and has a population of 1,033,381. Within SK there are 13 separate health regions, each with several hospitals; some with blood banks & others without. As a result, there are many challenges with ensuring patients have access to appropriate factor products, especially in rural areas.

Aims: The purpose of this study was to explore the challenges of effective distribution & management of factor over a large geographic area, and how these challenges can potentially be addressed.

Methods: This was a qualitative study using a phenomenology approach performed for program assessment purposes in 2012–13, that consisted of collecting stories describing challenges faced by health care professionals in transfusion medicine & the bleeding disorders program in SK.

Results: Currently in SK, 78 facilities are qualified to transfuse & 35 facilities hold blood product inventory. One of the main logistical challenges found was with respect to shipping product from the sole provincial Blood Center to hospitals as there is currently no provincial laboratory transport network.

Another major challenge identified was that of maintaining national transfusion standards in rural laboratories where staff are not specialists in blood banking & are unfamiliar with these specialty products.

Another issue identified was ensuring appropriate redistribution of factor products as they approach outdates. It is important that patients have access to factor, but it is also the duty of the provincial program to ensure that these expensive resources are being used appropriately with minimal wastage.

Lastly, a key to this entire system was found to be proper patient & health care community education. This is a crucial piece of the puzzle, especially when the medical team is often not able to assess patients in person as they are quite remote.

Conclusion: (Results & Discussion written together above) Poster will include: NEXT STEPS.

Disclosure of Interest: None declared.

TTP / thrombotic microangiopathies I

PO578-MON

Neuro-imaging does not correlate with disease severity in acute thrombotic thrombocytopenia purpura (TTP), but may predict long term sequelae

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Background: Neurological involvement is reported in up to 60% of patients presenting with TTP. The role of neuro-imaging in the acute setting and correlation with long term outcomes is not well defined.

Aims: To study neuro-imaging results in acute TTP patients and correlation with disease severity and residual neurological impairment.

Methods: A retrospective review was performed of patients with acute TTP during August 2013–December 2014 treated at a TTP Specialist Centre, $n = 13$. Data collection included: clinical presentation, critical care involvement, neuro-imaging modality and result, and long term neurological complications.

Results: 10/13 patients had neurological involvement at presentation: confusion 4/10, seizure 4/10, headache 1/10, and hemiparesis 1/10. 8/10 patients with neurological involvement required an ICU stay with 4/8 requiring invasive ventilation.

All 10 patients with neurology underwent imaging in the acute stages: 7/10 CT, 2/10 MRI, 1/10 MRI and MRA. 5/10 showed abnormalities of which 3/5 demonstrated ischaemic changes consistent with TTP, 1/5 age-related changes and 1/5 an incidental cyst. At a median follow up of 12 weeks (range 1–60 weeks), survival was 100%. 7/10 had resolving neurological impairment, however 3/10 patients with TTP-related neuro-imaging have required outpatient follow-up from other specialist teams: stroke/ophthalmology for persistent, significant neurological deficit.

Conclusion: Patients presenting with neurological manifestations of TTP are more likely to undergo neuro-imaging and require critical care support. Only a small proportion however, demonstrate abnormal imaging consistent with TTP indicating that treatment with plasma exchange should not be delayed to image these patients. Abnormal findings may predict long-term neurological disability requiring specialist support post-remission.

Disclosure of Interest: None declared.

PO579-MON

Phase II study of danazol with plasma exchange and corticosteroids for the treatment of thrombotic thrombocytopenic purpura

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Background: Danazol is effective in refractory thrombotic thrombocytopenia purpura (TTP). In 2009, we initiated a phase II study of danazol with plasma exchange (PE) and corticosteroids (CS) for the initial treatment of TTP. This trial was approved by the institutional review board and is registered at ClinicalTrials.gov. We have reached 50% of our accrual target and present an interim analysis.

Aims: To determine if danazol reduces number of PE's, time to remission (TR), and length of stay (LOS) when administered with standard therapy for the initial treatment of TTP.

Methods: Experimental Group (EG): Patients with TTP (Platelet count (PLT) < 100,000 μL^{-1} and microangiopathy receive daily PE, prednisone 1 mg kg^{-1} , and danazol 600 mg. Following remission (PLT > 150,000 μL^{-1} for 3 consecutive days), PE is tapered to every other day for 1 week then stopped. Then prednisone is tapered weekly over 4 weeks then stopped. After 1 week danazol is tapered by 200 mg weekly then stopped. Historical controls (HC): Patients treated for TTP at St. Luke's-Roosevelt Hospital Center with PE with or without CS from 2000 to 2007 ($n = 20$). Relapse definition: PLT < 100,000 μL^{-1} with microangiopathy > 30 days after remission. Total number of PE's, TR, LOS, and continuous response rates are compared between the 2 groups. Accrual target: 16 patients to detect a 40% decrease in the number of PE's with 80% power and a level of significance of 0.05.

Results: Eight patients have been enrolled. Average number of PE's (days) were 15 (EG) and 16.1 (HC), average TR (days) was 10.6 (EG) and 11 (HC), and average LOS (days) was 27.8 (EG) and 24.9 (HC), $P > 0.05$. Four patients in HC have had a total of 6 relapses at our institution at a median of 28.25 months, range 2.75 to 97 months after the initial event. No patients in EG have relapsed during a median follow-up of 25.5 months, range 6 weeks to 53 months.

Conclusion: Danazol does not appear to reduce PE requirement, TR, or LOS during initial therapy of TTP, but does appear to prevent relapse.

Disclosure of Interest: None declared.

PO580-MON

Dense genotyping of immune-related disease regions for the identification of genetic risk factors in acquired thrombotic thrombocytopenic purpura

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Background: Acquired thrombotic thrombocytopenic purpura (TTP) is associated with the development of autoantibodies against the VWF-cleaving protease ADAMTS13. Evidences of a genetic contribution have been reported, including the association of the human leukocyte antigen (HLA) complex with disease risk.

Aims: To identify novel genetic risk factors in acquired TTP.

Methods: A total of 195 Caucasian patients with acquired TTP were genotyped using the Illumina ImmunoChip. Enrolled cases presented a diagnosis of TTP (episode of thrombocytopenia, microangiopathic hemolytic anemia without alternative causes) and anti-ADAMTS13 antibodies by western blot. Cases were compared with 1255 Italian controls using logistic regression models. A replication study using SNP TaqMan assays is ongoing to validate the associated variants. So far, 47 cases and 149 controls have been tested.

Results: After quality control 186 cases, 1255 controls and 131095 variants were available for association testing, with a total genotyping rate of 0.99993. Logistic regression analysis revealed 7 associated variants, of which the strongest variant, rs6903608 ($P = 1.51 \times 10^{-14}$), was identified within the HLA class II complex, with a minor allele frequency (MAF) of 0.498. We also identified a rare variant, rs115265285 (MAF=0.006, $P = 3.20 \times 10^{-5}$), located 3.5 kb into the 3'UTR of the *DNMT3A* gene. All variants presented strong effects and were associated with either elevated risk of disease, showing odds ratios (OR) ranging from 2.13 to 7.53, or protective effect, with ORs of 0.39 and 0.59. In the replication study, preliminary data confirmed the association of rs6903608 with the disease ($P = 4.4 \times 10^{-3}$).

Conclusion: Our study has confirmed the association of the HLA locus with the risk of developing a TTP episode. Replication on other non-HLA variants, which requires a larger sample size, is currently ongoing.

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PO581-MON

Characterization of single sorted anti-ADAMTS13 specific B cells from the spleen of acquired thrombotic thrombocytopenic purpura (ATTP) patients

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Background: Autoantibodies (Abs) that neutralize and/or accelerate the clearance of ADAMTS13 cause aTTP. Increasing evidence points at the spleen as a major reservoir for antigen specific memory B-cells.

Aims: We investigated the B-cell population and frequency of ADAMTS13-specific B-cells in the spleen of aTTP patients to understand the humoral autoimmune response and to develop an Abs/B-cells targeted therapy.

Methods: Splenic mononuclear cells of 7 aTTP patients- 2 treated with Rituximab (RTX) were analysed by flow cytometry. The frequencies of highly positive anti-ADAMTS13 B-cells among naïve, un-, switched memory B-cells and plasma cells were determined. Individual B-cells based on binding to fluorescently labelled rADAMTS13 were sorted. Gene transcripts of single cells were reverse-transcribed followed by nested PCR to amplify IgG heavy/light chain genes and sequencing. Identifying the Ig variable domains (V) and V-(D)-J junctions i.e. antigen binding region (CDR3) was performed using IMIGT/V-QUEST tool.

Results: Splenic anti-ADAMTS13 cells were detected in all patients (0.057% of total B-cell population) with highest prevalence among plasma cells. The 2 RTX treated patients had decreased levels of CD19/CD20 positive cells (1% and 20% of lymphocytes after T-cell/monocyte exclusion), compared to RTX- naïve (~63%). Splenic anti-ADAMTS13 specific B-cells of 4 aTTP patients revealed 83 Abs from which CDR3 diversity was analysed. Most frequently used V-genes were IGHV1-69, IGHV3-30, IGHV5-51 (15%, 12%, 8%). Abs had average germline identity of 92.2% with 17 amino acids CDR3 region. Igk light chain was used by 41 Abs vs. 30 Abs were Igλ. We detected two cells bearing Abs which were positive for both: Igκ+, Igλ+.

Conclusion: Specific anti-ADAMTS13 B-cells were found in the spleen of all aTTP patients, including plasma cells known to be unaffected by RTX. Currently we clone selected monoclonal Abs. Functional testing will allow selection of the inhibitory Abs as tools to develop antigen-specific therapies for aTTP.

Disclosure of Interest: None declared.

PO582-MON

The natural mutation ASP173GLY in the catalytic site of the ADAMTS-13 gene causes a severe Upshaw-Schulman syndrome: clinical course, biochemistry and molecular dynamics investigation

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Background: Congenital thrombotic thrombocytopenic purpura (TTP), also referred to as Upshaw-Schulman syndrome, is a rare form of thrombotic microangiopathy, caused by a dysfunction or severe deficiency of ADAMTS-13 (A Disintegrin And Metalloprotease with ThromboSpondin 1 repeats), a zinc-protease which physiologically is responsible for the proteolytic processing of VWF multimers. The disease is inherited with autosomal recessive mode and caused by mutations in the ADAMTS-13 gene. About 100 mutations of the ADAMTS-13 gene were identified so far, although only a few characterized by *in vitro* expression studies.

Aims: Study a new Asp to Gly homozygous mutation at position 173 of ADAMTS-13 sequence, which was identified in a family of Romanian origin, with some members affected by clinical signs of TTP.

Methods: Expression studies were carried out in mammalian cells with a complete biochemical and molecular dynamics investigation of the D173G mutation.

Results: In two male sons this mutation caused a severe (<1%) deficiency of ADAMTS-13 activity and antigen level, associated with periodic thrombocytopenia and haemolytic anemia. Both parents, who are cousins, showed the same mutation in heterozygous form. The mutant enzyme was expressed in HEK293 cells, but showed a severe decrease of secretion and detectable levels inside the cell. Molecular dynamics found that in the D173G mutant the interface area between the metalloprotease domain and the disintegrin-like domain significantly decreases from 1090 to 741 Å² during the simulations, while the proline-rich 20 residues linker region (LR, 285–304) between them undergoes extensive conformational changes. Inter-domain contacts are also significantly less conserved in the mutant compared to the wild-type.

Conclusion: These results may help to unravel the role of the mutation in the maturation and folding of ADAMTS-13, likely destabilizing the enzyme and impairing its conformation and secretion.

Disclosure of Interest: None declared.

PO583-MON

Defective degradation of neutrophil extracellular traps is associated with acute thrombotic microangiopathies

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Background: Thrombotic Microangiopathies (TMAs) have been associated with neutrophil extracellular traps (NETs). NETs consist of extracellular DNA fibers decorated with histones and proteases, which bind and activate platelets as well as clotting factors.

Aims: We hypothesized that efficient removal of NETs is necessary to inhibit excessive thrombosis. Therefore, we investigated whether acute TMAs are associated with a defect in the degradation of NETs.

Methods: NETs produced *in vitro* were incubated with plasma from TMA patients (*n* = 27) and from healthy controls (*n* = 10). DNase1 activity was measured in the plasma samples by single radial enzyme diffusion assays, as well as denaturing PAGE zymograms.

Results: Our data shows that NETs exposed to plasma from healthy donors were efficiently degraded, whereas NETs remained stable after exposure to plasma from TMA patients (*P* < 0.01). The inability to degrade NETs strongly correlated with a decreased DNase1 activity in plasma (*r* = -0.855). We found that this lower activity of the enzyme is not due to the presence of inhibitors, but to a decreased amount of the active enzyme in circulation. Supplementation of plasma from TMA patients with recombinant human DNase1 successfully restored the NET-degrading activity.

Conclusion: NET-degrading activity mediated by DNase1 is impaired in patients with TMA. The role of plasma DNases in thrombosis is poorly understood. Reduction in the circulating DNase1 activity may cause persistence of pro-thrombotic NETs, and promote microvascular thrombosis in TMA patients.

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PO584-MON

Platelet inhibition in TTP: a mechanism to escape thrombosis

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Background: Thrombotic Thrombocytopenic Purpura (TTP) is a severe thrombotic disorder that mainly affects young women and has a mortality rate of seventy percent if untreated. It is caused by the spontaneous binding of ultralarge multimers of the plasma protein Von Willebrand Factor (VWF) to circulating platelets, leading to microvascular occlusions in organs such as the brain and the kidney. Patients with acquired TTP may be in remission for prolonged periods of time, despite the continued absence of functional ADAMTS13 and the presence of ultralarge VWF. The mechanism behind the intermittent pattern of attacks in TTP is currently unclear. We hypothesized that platelets may play a modulating role.

Aims: To study platelet function in TTP patients, in remission: Methods: We have studied platelet VWF interaction and platelet function in 30 TTP patients who are in remission and in 20 healthy controls. Furthermore, we measured ADAMTS-13 activity and VWF multimer patterns. Finally, we reconstituted platelets from normal volunteers in plasma of TTP patients to study whether TTP plasma affects platelet function.

Results: We observed spontaneous binding of VWF to platelets in TTP patients, which does not occur in normal controls. Binding of VWF to platelets was inversely correlated with platelet count. As expected, the TTP patients had more ultra large VWF than the healthy controls. Furthermore, platelet activation by ADP and TRAP-6 is strongly inhibited in TTP patients as compared with healthy controls. This inhibition of platelet activation was confirmed by reconstitution experiments, in which plasma of TTP patients were added to platelets of healthy controls.

Conclusion: TTP patients in remission have spontaneous VWF binding and a reduced sensitivity to platelet activation by P2Y12 receptor agonists or by thrombin receptor agonists. This platelet inhibition may be an escape mechanism for severe thrombotic complications.

Disclosure of Interest: None declared.

PO585-MON

Treatment of a Jehovah's witness with recurrent thrombotic thrombocytopenic purpura with Rituximab

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Background: Thrombotic thrombocytopenic purpura (TTP) is a potentially fatal disease characterized by microangiopathic hemolytic anemia, thrombocytopenia, and a variable degree of organ dysfunction, especially renal failure and neurological disease and is often refractory to treatment with high relapse rate. Daily therapeutic plasma exchange is the mainstay of treatment. The management of patients with TTP whose religious beliefs preclude the use of blood or blood products can often be difficult and lead to fatal results.

Aims: To describe a case of successful treatment of a Jehovah's witness with recurrent TTP: Methods: A 58-year-old African-American female presented with TIA-like episode of aphasia and blurring of vision. Her labs were significant for platelet count of 30,000 μL^{-1} , Hemoglobin of 9.1 gm dL⁻¹, LDH 756 U L⁻¹ and Creatinine of 1.1 mg dL⁻¹, with

latest normal lab values, almost 3 months ago. Originally, she came to us two years ago with similar presentation with labs significant for Platelet count 16,000 μL^{-1} , Creatinine 1.2 mg dL⁻¹, Haptoglobin ≤ 10 mg dL⁻¹, LDH 1603 U L⁻¹, Hemoglobin 5.4gm dL⁻¹, Reticulocyte count 6.8% and peripheral blood smear showing many schistocytes, leading to a diagnosis of TTP. Patient refused plasmapheresis or transfusion of blood products and was successfully treated with Rituximab and Cyclophosphamide with significant clinical improvement and improved laboratory parameters; a platelet count of 248,000 μL^{-1} and hemoglobin of 8.4 gm dL⁻¹. The current relapse was also treated in similar fashion with Rituximab and IVIG.

Results: MRI showed multiple tiny acute ischemic changes in the left middle cerebral artery distribution, likely due to the current relapse of TTP. She showed significant improvement within few days.

Conclusion: Rituximab appears to be safe and effective, targeted therapy, especially in patients, where conventional treatment can not be initiated due to various reasons, including religious beliefs. Its true role in long term is still to be ascertained.

Disclosure of Interest: None declared.

PO586-MON

Pathogenicity of anti-ADAMTS13 autoantibodies in acquired TTP

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Background: Acquired thrombotic thrombocytopenic purpura (TTP) is an autoimmune disease in which anti-ADAMTS13 autoantibodies (auto-Ab) cause severe enzyme deficiency with ADAMTS13 activity $<10\%$.

Aims: To characterise the domain specificity and pathogenic mechanisms of anti-ADAMTS13 auto-Ab in acquired TTP.

Methods: We analysed 92 acquired TTP episodes at presentation through treatment and remission/relapse using epitope mapping and functional analyses.

Results: 89/92 (97%) episodes had auto-Ab recognising the ADAMTS13 N-terminal domains (MDTCS). The spacer domain was the only N-terminal antigenic target detected. 38/92 (41%) episodes had auto-Ab recognising N-terminal domains alone; 54/92 (59%) had antibodies against the C-terminal domains (26/92 against TSR2-8 domains and 28/92 against CUB domains). Changes in auto-Ab specificity were evident at relapse in 9/16 relapse patients. Functional analyses on IgG from 43 patients revealed inhibitory IgG were limited to anti-spacer domain antibodies. However, 15/43 patients had autoantibodies with no detectable inhibitory action, suggesting a different pathogenic mechanism in these patients. We therefore analysed plasma ADAMTS13 antigen levels. All 91 acquired TTP presentation samples exhibited markedly reduced ADAMTS13 antigen levels, median 6% normal (range 0–47%), ADAMTS13 antigen in the lowest quartile ($<1.4\%$) at first presentation was associated with increased mortality (odds ratio 5.7).

Conclusion: Anti-spacer domain autoantibodies are the major inhibitory antibodies in acquired TTP. However, depletion of ADAMTS13 antigen (rather than enzyme inhibition) is the prevailing pathogenic mechanism in acquired TTP, and ADAMTS13 antigen levels at presentation have prognostic significance. Taken together, our results provide new insights into the pathophysiology of acquired TTP. Understanding the kinetics of antibody-mediated clearance of ADAMTS13 may provide opportunities for new treatment modalities to be developed

Disclosure of Interest: None declared.

PO587-MON

Major clinical outcomes are similar between initial and relapsed episodes of thrombotic thrombocytopenic purpuraYang S¹, Geyer S², Biller E¹, Wu H¹, Cataland SR³ and Wu HM¹¹Pathology, Ohio State University, Columbus; ²Epidemiology, University of South Florida, Tampa; ³Internal Medicine, Ohio State University, Columbus, USA

Background: About 35% of TTP patients developed relapse episodes after initial presentation. However, it is unclear if clinical and laboratory characteristics of relapses are significantly different from those in the initial event. The data is important for guiding clinicians in managing TTP patients and to determine if it is appropriate to include the relapsed episode into therapeutic trials.

Aims: Comparing clinical outcomes between initial (I) and relapsed (R) episodes of TTP.

Methods: Between 2003 and 2014, OSU-TTP program treated 127 episodes of acute TTP from 67 patients. Of these, 49 were seen at OSU for their initial and subsequent TTP episodes. The other 18 were referred and treated at OSU for relapsed event(s). Laboratory/demographic data and treatment outcomes were analyzed in both I ($n = 49$) and R ($n = 78$) episodes.

Results: There were no significant differences in the main clinical outcomes between the I and R groups in rates of treatment success ($P = 0.8$), exacerbation ($P = 0.9$), refractory ($P = 0.4$), mortality ($P = 0.35$), and in number of plasma exchange (PE) required to achieve a clinical response ($P = 0.7$). Significant differences were not seen in a majority of laboratory markers including creatinine, Hg, ADAMTS13 inhibitor, IgG, antigen levels, and complement markers (C4d, C3a, C5a, C5b9, and Bb). However, I group showed severe laboratory abnormalities in the platelet ($I < R$, $P < 0.01$), LDH ($I > R$, $P < 0.01$), and ADAMTS13 activity ($I < R$, $P = 0.04$). R group is significantly younger than I group ($P < 0.01$). When examining the impact of immunotherapy (IT), the patients who developed relapse while on recent IT ($n = 23$) had a significantly lower rate of treatment success, more PEs required, and higher exacerbation/relapse rate than the control events ($n = 92$).

Conclusion: The major clinical outcomes between I and R episodes of TTP are similar. For clinical trials, our data supports the strategy to enroll patients with a relapsed event of TTP. However, the history of IT must be considered as a major confounder during analysis.

Disclosure of Interest: None declared.

PO588-MON

Activation of the complement system and reduction of ADAMTS13 activity both precede clinical events of TTP relapseYang S¹, Cataland SR², Wu H¹, Jin M¹, Wang Y³, Lundberg AS³ and Wu HM¹¹Pathology; ²Internal Medicine, Ohio State University, Columbus; ³Alexion Pharmaceutical Inc, Cheshire, USA

Background: About 35% of patients with TTP experience clinical relapse. There is a strong clinical need for biomarkers to monitor disease relapses and to treat patients preemptively.

Aims: Exploring the biomarkers predicting TTP relapse.

Methods: The OSU-TTP program has been following all TTP patients throughout clinical remission with samples collected quarterly for study until relapse occurred. 42 pre-relapse samples, defined as the blood collected within 3 mo prior to TTP relapse, were obtained from 23 patients who had one or several subsequent relapses. Complement biomarkers and ADAMTS13 activities were analyzed and compared to the control samples from non-relapsers ($n = 19$) and the controls

from relapsers ($n = 31$) obtained from quarterly visits without a relapse in the subsequent 3 mo.

Results: Complement biomarker and ADAMTS13 levels in pre-relapse samples were significantly different from both control groups. The median C3a (816 vs. 187 ng mL⁻¹) and C5b-9 (440 vs. 265 ng mL⁻¹) levels were significantly elevated in pre-relapse samples ($P < 0.05$ in both). In contrast, the ADAMTS13 levels were significantly reduced ($P < 0.01$, median 5% vs 43%). By ROC analysis, ADAMTS13 activity at $<20\%$, C5b-9 at >310 ng mL⁻¹, or C3a at >300 ng mL⁻¹, would correctly predict a clinical event of relapse in approximately 70% of cases. When the pre-relapse samples were further analyzed according to the time to relapse (i.e. 8–12 weeks, 4–8 weeks, 2–4 weeks, and <2 weeks), C3a, C5a, C5b-9, and factor Bb showed a clear trend of elevation toward the time point of relapse with the highest values seen at the interval of <2 weeks. However, ADAMTS13 activity level was reduced to the lowest level 2–4 weeks prior to relapse.

Conclusion: Complement activation and ADAMTS13 activity are useful biomarkers to predict events of TTP relapse during period of clinical remission. The time-course study suggests that a significant fall in ADAMTS13 activity may be a driving event that leads up to ULVWF, thrombosis, complement activation and ultimately a TTP relapse.

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PO589-MON

Baseline demographics and characteristics of 570 patients with atypical hemolytic uremic syndrome in the global aHUS registryLicht C¹, Ardisino G², Ariceta G³, Cohen DJ⁴, Gasteyger C⁵, Greenbaum LA⁶, Johnson S⁷, Ogawa M⁸, Kupelian V⁸, Schaefer F⁹, Walle JV¹⁰ and Fremaux-Bacchi V¹¹¹The Hospital For Sick Children, Toronto, Canada; ²Fondazione

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Background: Atypical hemolytic uremic syndrome (aHUS) is a rare, genetic, life-threatening disease mostly caused by chronic, uncontrolled complement activation that leads to thrombotic microangiopathy and renal and other end-organ damage. The aHUS Registry, established in April 2012, is an observational, non-interventional, multicenter, global registry designed to collect information on patients (pts) with aHUS. By facilitating the availability of the follow-up data on the aHUS indication for eculizumab (ECU), the Registry fulfills postmarketing regulatory requirements while also highlighting the need for and benefit of a sponsor/academia partnership.

Aims: To report baseline demographics and clinical characteristics of pts enrolled in the Registry as of December 18, 2014.

Methods: Pts of all ages with a clinical diagnosis of aHUS (irrespective of treatment) are eligible for enrollment. An identified complement abnormality is not required. Demographic, medical and disease his-

tory, treatment, and efficacy and safety outcomes data are collected at Registry enrollment and prospectively thereafter.

Results: By December 18, 2014, 570 pts were enrolled from 16 countries; 351 (61.6%) were ≥ 18 years of age. Mean (standard deviation) age at initial symptoms and diagnosis was 22.4 (20.6) and 22.9 (20.6) years, respectively. Family history of aHUS was reported by 106 pts (18.6%). Prior to enrollment, plasma exchange/plasma infusion, dialysis, and renal transplantation were reported by 340 (59.6%), 334 (58.6%), and 109 pts (19.1%), respectively. ECU was administered to 303 pts (53.2%) before enrollment.

Conclusion: Following public disclosure of data from the last aHUS Registry update, pt enrollment has continued to increase. Ongoing and future analyses will further enhance understanding of the history and progression of aHUS. Additional clinical sites are encouraged to enroll pts to facilitate knowledge acquisition and optimization of pt care and quality of life.

Disclosure of Interest: C. Licht Grant/Research Support from: Alexion Pharmaceuticals, Inc., Consultant for: Alexion Pharmaceuticals, Inc., G. Ardisino Consultant for: Alexion Pharmaceuticals, Inc., G. Ariceta Grant/Research Support from: Alexion Pharmaceuticals, Inc., Consultant for: Alexion Pharmaceuticals, Inc., D. Cohen: None Declared, C. Gasteyger Shareholder of: Alexion Pharma International SàRL, Employee of: Alexion Pharma International SàRL, L. Greenbaum Grant/Research Support from: Alexion Pharmaceuticals, Inc., Consultant for: Alexion Pharmaceuticals, Inc., S. Johnson Grant/Research Support from: Alexion Pharmaceuticals, Inc., M. Ogawa Shareholder of: Alexion Pharmaceuticals, Inc., Employee of: Alexion Pharmaceuticals, Inc., V. Kupelian Shareholder of: Alexion Pharmaceuticals, Inc., Employee of: Alexion Pharmaceuticals, Inc., F. Schaefer Consultant for: Alexion Pharmaceuticals, Inc., J. Vande Walle Grant/Research Support from: Alexion Pharmaceuticals, Inc., Consultant for: Alexion Pharmaceuticals, Inc., Speaker Bureau of: Alexion Pharmaceuticals, Inc., V. Fremaux-Bacchi Consultant for: Alexion Pharmaceuticals, Inc.

PO590-MON

A case of thrombotic microangiopathy with CFH gene alterations that are not associated with atypical hemolytic uremic syndrome

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Background: Atypical hemolytic uremic syndrome (aHUS) is a rare disorder characterized by thrombotic microangiopathy (MAHA). Atypical hemolytic uremic syndrome is a mutigenic disorder. Among the genes that are associated with aHUS, CFH gene mutations are the most common genetic cause of the disease. There are several specific gene mutations identified in the aHUS patients. In this study, we present a case with MAHA symptoms.

The patient is a five-years-old boy. He had clinics of MAHA. He had microangiopathic hemolysis with 10% schistocytes and polychromasia in the blood smear. According to blood smear and other MAHA symptoms, firstly plasmapheresis was started for 20 weeks. Then treatment was continued with eculizumab (once a week, 600 mg). Although the patient had MAHA symptoms, he didn't have renal insufficiency. Therefore, in order to confirm clinical the diagnosis CFH gene analysis was required.

Aims: The purpose of the study was to perform CFH gene mutation analysis to confirm the clinical diagnosis.

Methods: CFH gene was screened for a mutation by direct DNA sequencing analysis of the CFH gene.

Results: ADAMTS13 activity was normal, the antibody was negative. DNA sequencing analysis revealed that the patient is homozygous for p.His402Tyr, heterozygous for p.Ala307Ala and p. Ala473Ala SNPs in the CFH gene.

Conclusion: CFH gene variations that were identified in our case have not been associated with aHUS. P.His402Tyr is located in the functional domain of the protein and previously identified in aHUS patients together with a specific CFH gene mutations. On the other hand, these polymorphisms were only CFH gene variations identified without any specific CFH gene mutations associated with aHUS. P.His402Tyr has been associated with age related macular degeneration as well. Since the location of the P.His402Tyr in the functional domain of the protein, it might have a pathogenic effect for aHUS patients. Therefore, larger aHUS patient population should be screened for p.His402Tyr SNP to validate its pathogenicity.

Disclosure of Interest: None declared.

PO591-MON

Severe first manifestation of TTP in a 96-year-old female

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Background: Middle age and female gender are well-known risk factors for acquired TTP. **Aims:** We report the case of a 96-year-old, very spry and independently-living female who came to her general practitioner because of suddenly developed anemia and epistaxis.

Methods: Laboratory findings: thrombocytopenia of 5.900, Hb of 7.1 g dL⁻¹, schistocytes 57 % and an increased LDH level of 436 U L⁻¹. 3 weeks earlier, all blood values had been found to be normal when examined by the general practitioner.

Results: Because of strongly suspected TTP, the patient was immediately admitted to our hospital.

Supplementary laboratory values:

Liver values: regular

Creatinine: increased to 1.6 mg dL⁻¹ (normal value: < 1.1 mg dL⁻¹)

Troponin: increased to 5388 pg mL⁻¹ (normal value: < 24 pg mL⁻¹)

ADAMTS 13 activity: 1.2% (normal value: > 50%)

ADAMTS 13 inhibitor: 1.8 BU

The patient was instantly treated with plasma exchange. Cerebral haemorrhage as well as ischaemia and cerebral oedema could be excluded after repeated CCTs, also a cardiac infarction.

Pre-existing conditions: Paroxysmal atrial fibrillation and a well-controlled hypertension.

Medication: Phenprocoumon (INR 1.41 at admission), Ranolazin 375 mg, Torasemid 5 mg.

Conclusion: Our 96-year-old patient suffered from a sudden TTP bout. This case report demonstrates that TTP can also occur in very old persons. To the best of our knowledge, this is the first published case report on TTP in a very old patient.

Disclosure of Interest: None declared.

PO592-MON

Genotype and phenotype features of congenital thrombotic thrombocytopenic purpura patients (milan TTP registry)

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Background: Congenital thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening disease due to mutations in *ADAMTS13* gene.

Aims: To describe the clinical severity of congenital TTP patients referred to our centre from 2002 to 2014.

Methods: Patients with a diagnosis of congenital TTP and mutations on *ADAMTS13* gene were identified from the Milan TTP registry. Data were collected using a standardized questionnaire. Signs and symptoms of acute TTP were classified into 3 major categories: bleeding events, neurological and renal involvement. Patients with available plasma collected during remission at least 15 days from the last fresh frozen plasma (FFP) infusion were tested for ADAMTS13 residual activity using a highly sensitive method (SELDI-TOF-MS). The association of ADAMTS13 activity with clinical presentation was studied by regression analysis.

Results: Data on 21 unrelated congenital TTP patients were analyzed. We identified 30 mutations (22 missense, 6 Ins/del, 1 splice site, 1 nonsense), 8 of which novel. Median age at disease onset was 7 years (range: 0–55 years), with 15 (71%) patients manifesting recurrent TTP episodes. Neonatal jaundice/thrombocytopenia were reported in 8 (38%) patients. Bleeding events were observed in 16 (80%) patients, with 2 (10%) patients presenting intracranial hemorrhage, neurological involvement in 12 (60%) and renal involvement in 10 (50%). Fever, jaundice, abdominal pain and vomiting/diarrhea were present in 12 (60%), 8 (40%), 5 (25%) and 4 (20%) patients. Twelve (57%) patients were on FFP prophylaxis. Median ADAMTS13 residual activity was 0.9% (range: <0.5–5.9%, $n = 13$). Regression analysis revealed a potential association of residual ADAMTS13 activity with platelet count (beta 2.5, 95% CI –0.03–5.06) and occurrence of bleeding events (OR 0.37, 95% CI 0.12–1.12).

Conclusion: Clinical presentation in congenital TTP is highly variable. Higher levels of residual ADAMTS13 activity seemed to be associated with higher platelet count and decreased occurrence of bleeding events.

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PO593-MON

Neurological and psychological consequences after recovery from thrombotic thrombocytopenic purpura (TTP)

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Background: Neurological and psychological abnormalities are common in patients who have experienced an acute TTP episode. In TTP phases of acute illness alternate with relatively symptom-free phases of remission.

Aims: Aim of the study was to investigate the risk of a cognitive deficit, depression and an anxiety disorder in consequence of TTP.

Methods: One hundred TTP patients were analyzed twice in a two year period or at time of the initial diagnosis by means of different questionnaires. We used 2 standardized questionnaires to evaluate depression (IDS-SR, Inventory of depressive symptomatology-self-report) and to examine mental performance (FLei, Fragebogen zur subjektiven Einschätzung der geistigen Leistungsfähigkeit). Additionally we developed a TTP-specific anxiety questionnaire.

Results: Seventy percent of the patients suffered from depression. At the time of consultation only 7.2% received psychotherapy. Patients who had their last TTP episode within the past year suffer obviously more from depression than patients with no bout in the past year ($P = 0.055$). In contrast, there is no correlation between depression and the frequency or severity of the acute episode. Mental performance (executive function, memory, attention) correlates strongly with the severity of depression (Spearman: 0.696). For controls and patients with depression our results are in accordance with the literature. In a separate study we developed a specific questionnaire (TAF) to analyze anxieties of TTP patients. TAF was validated in 120 controls describing anxiety during daily life, treatment, acute TTP bouts and other diseases. The TTP collective exhibited significant more anxiety disorders compared to the control group.

Conclusion: TTP patients suffer from depression, anxiety disorders as well as cognitive disturbances, especially during the first year after recovery of an acute episode. Furthermore, we assume that the cognitive disturbances are the consequence of depression and anxiety, independently of TTP episodes.

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Venous thromboembolism – diagnosis I

PO594-MON

The new clinical prediction score for diagnosis of lower limb deep vein thrombosis in unselected population of outpatients and inpatients

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Background: The modified Wells score and the Wells score could be useful in prediction of deep vein thrombosis (DVT) in the outpatients;

however, the accuracy of the scores in the unselected population of outpatients and inpatients was inaccurate.

Aims: To develop a new clinical prediction score for diagnosis of lower limb DVT in unselected population of outpatients and inpatients.

Methods: We developed a new score by multivariate analysis of consecutive 500 outpatients and inpatients with clinically suspected of lower limb DVT addressed to the DVT consultation. The new score was validated and compared with the other score in the new consecutive 315 outpatients and inpatients.

Results: From the derivation sample of 500 outpatients and inpatients, the new four-component score was developed as follows: unilateral lower limb pain (+1), confinement to bed more than three days(+1), calf enlargement of more than 3 cm compared to the other sites(+1), and previous venous thromboembolism(+1). A total score of 2 or more meant high probability while a score less than 2 meant low probability for DVT. In the validation sample of 315 outpatients and inpatients, the new score identified 106 patients who had a high probability of DVT, of whom 58 (54.7%) had DVT. In 209 patients who had a low probability, of whom 23 (11.0%) had DVT. The area under the ROC curve was 0.79 (95% confidence interval [CI]: 0.74–0.85) for the new score. A normal D-dimer result had a negative predictive value of 96.43% (95% CI: 81.65% – 99.91%) for the exclusion of DVT when there is low probability for the DVT by the new score.

Conclusion: Our new four-component prediction score might be useful in unselected population of outpatients and inpatients being evaluated for DVT. When using the new score with D-dimer test, negative D-dimer test in low probability group could be the rule out test for DVT in unselected population of outpatients and inpatients.

Disclosure of Interest: None declared.

PO595-MON

Over-representation of severe subclavian vein occlusion in the affected arm, compared with the contralateral arm, in patients with unprovoked unilateral upper extremity deep vein thrombosis

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Background: It is controversial whether subclavian vein occlusion in the thoracic outlet is the etiology of upper extremity deep vein thrombosis (UEDVT) because normal persons may have similar radiological abnormalities. Since most patients only presented with unilateral arm thrombosis, the contralateral non-thrombotic arm provides a paired control to examine the causative association between UEDVT and subclavian vein occlusion in the costoclavicular (CC) space.

Aims: With bilateral contrast-enhanced 3D magnetic resonance angiography (cMRA), we evaluated the arms in provocative posture and then compared the affected arm with one's own contralateral arm for the degree of vessel occlusion in patients with UEDVT after the acute thrombus has resolved.

Methods: Eight patients with unprovoked UEDVTs from 2012 to 2014 were consecutively imaged with cMRA in abducted and adducted positions. No intraluminal thrombus was noted. The distance between the cortex of the 1st rib to the underside of the subclavius muscle was defined as CC space. From each side in each position, the following four parameters were measured: vein caliber, artery caliber, CC space for vein (CCv), and CC space for artery (CCa).

Results: In resting (adducted) position, none of the patients had complete occlusion of the blood vessels. However, all patients had narrower CC spaces (55% – 82% reduction) or smaller vessel diameters (56% – 100% less) in abducted position, consistent with bilateral thoracic outlet obstruction. The vein caliber in all patients had either 100% occlusion ($n = 7$) or more severe compression than the control

arm ($n = 1$). Yet, only 4 patients had complete occlusion of the vein in the control arm and none of the CCv, CCa, nor artery caliber had 100% occlusion, either in the lesion or the control arm.

Conclusion: Complete compression of subclavian vein in the abducted arm having UEDVT is more common than the contralateral, non-affected arm suggesting a causative association between venous compression and the development of thrombosis.

Disclosure of Interest: None declared.

PO596-MON

The performance of the age-adjusted d-dimer threshold for suspected pulmonary embolism in relevant subgroups: an individual patient data meta-analysis of 7000 patients

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Background: It has recently been demonstrated that pulmonary embolism (PE) can safely be excluded based on the combination of a Wells score indicating 'PE unlikely' and a D-dimer below an age-adjusted D-dimer threshold (age $\times 10$ in $\mu\text{g L}^{-1}$) in patients older than 50 years (Righini et al., JAMA, 2014). However, it is unknown whether the performance also applies to relevant subgroups.

Aims: To evaluate the performance of the age-adjusted D-dimer threshold overall and in subgroups of patients with COPD, age ≥ 75 years, cancer, previous VTE, and inpatients.

Methods: Individual patient data were used from 5 large prospective studies in which the diagnostic management of PE was guided by the Wells rule and D-dimer testing. A random effects meta-analysis was performed to calculate the performance of the age-adjusted D-dimer threshold in excluding PE in patients > 50 years with an unlikely Wells score, compared against the conventional threshold, overall and in the predefined subgroups.

Results: The dataset comprised 7027 patients of whom 4527 (64.4%) were older than 50 years. The PE prevalence in these patients was 26.8%. In patients > 50 years with a Wells score indicating 'PE unlikely', PE could have been excluded in 25% with the age-adjusted D-dimer threshold compared to 18% with the conventional D-dimer threshold (absolute increase 6.7%, 95% CI 4.5–9.7%). This performance was most prominent in patients with COPD (12%, 95% CI 9–15%) and elderly patients (13%, 95% CI 8–20%), and modest in patients with cancer (6%, 95% CI 4–8%), previous VTE (4%, 95% CI 2–7%), and inpatients (4%, 95% CI 3–6%).

Conclusion: Applying the age-adjusted D-dimer threshold increases the proportion of patients with suspected PE that can be managed without CT-scanning by approximately one third. The performance of an age-adjusted D-dimer cut-off is highest in patients with COPD and elderly patients, and less prominent in patients with cancer, previous VTE, and inpatients.

Disclosure of Interest: None declared.

PO597-MON

A simple decision rule including the D-dimer test to reduce the need for CT-scanning in patients with suspected pulmonary embolism

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Background: An unlikely clinical decision rule with a negative D-dimer test result safely excludes pulmonary embolism (PE) in 30% of presenting patients.

Aims: To simplify this diagnostic approach and to increase its efficiency.

Methods: Data of 723 consecutive patients with suspected PE were analysed. After constructing a logistic regression model with the D-dimer test result and items from Wells' rule, we identified the most prevalent combinations of influential items and selected new D-dimer positivity thresholds. The performance was validated in a dataset including 2785 consecutive patients with suspected PE.

Results: Three Wells items significantly added incremental value to the D-dimer test (22% PE prevalence): haemoptysis, signs of deep vein thrombosis and 'PE most likely'. Based on the most frequent combinations of these three items, we identified two groups: (1) none of these three items positive (41%), (2) one or more of these items positive (59%). When applying a 1000 µg L⁻¹ D-dimer threshold in group 1 and 500 µg L⁻¹ in group 2, PE could be excluded without CT-scanning in 36%, at a false-negative rate of 1.2% (95%: 0.04–3.3%). In the validation set, these proportions were 46% and 1.9% (95% CI 1.2–2.7%), respectively. Using the conventional Wells rule with a normal D-dimer result, these rates were respectively 22% and 0.6% (95% CI 0.10–2.4%).

Conclusion: Combining Wells items with the D-dimer test resulted in a simplified decision rule, which reduced the need for CT-scanning in patients with suspected PE. A prospective evaluation is required before it can be implemented in clinical practice.

Disclosure of Interest: None declared.

PO598-MON

Accuracy of X-ray with perfusion scan in young patients with suspected pulmonary embolism

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Background: Computed tomography pulmonary angiogram (CTPA) has become the standard test in the diagnostic workup of patients with suspected pulmonary embolism (PE). However, young patients may have an increased risk of cancer with CTPA. Perfusion scanning com-

bined with chest X-ray (X/Q) may offer an adequate alternative, but has never been prospectively validated.

Aims: To directly compare this X/Q strategy with CTPA in patients aged ≤50 years with suspected PE.

Methods: Consecutive patients with a likely clinical probability or an abnormal D-dimer level underwent both CTPA and X/Q. Two trained and experienced nuclear physicians independently analyzed the X/Q-scans. The accuracy of X/Q according to the PISAPED criteria was calculated in terms of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Results: Seventy-six patients were included, with a PE rate of 33%. The inter-observer agreement for X/Q-scan reading was high (κ=0.89). After consensus reading, 21 patients (28%) were categorized as 'PE present', 53 (70%) as 'PE absent', and two (2.6%) as 'non-diagnostic'. In 22% was a discrepancy between the X/Q-scan and CTPA. The PPV and NPV were 71% and 83%, respectively.

Conclusion: In patients with a high risk of PE, a diagnostic strategy of chest X-ray and perfusion scanning using the PISAPED criteria seems less safe than CTPA.

Disclosure of Interest: None declared.

PO599-MON

Development of elisa system for detection of protein S K196E mutation, a genetic risk factor for venous thromboembolism

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Background: Protein S (PS) is an anticoagulant protein that acts as a cofactor for activated protein C. PS K196E (Lys196 to Glu, Tokushima) mutation is a prevalent genetic risk factor for venous thromboembolism in a Japanese population, with odds ratio between 3.7 and 8.5. The E-allele frequency is approximately 0.9% in the Japanese population. Because of the substantial overlap in PS anticoagulant activity between wild-type (KK) and heterozygous K196E (KE) genotypes of PS, it is difficult to identify K196E carriers by measuring PS anticoagulant activity.

Aims: We sought to generate monoclonal antibodies specific for the PS K196E mutant and develop a sandwich enzyme-linked immunosorbent assay (ELISA) for the identification of PS K196E carriers.

Methods: We immunized GANP[®] mice with a keyhole limpet hemocyanin-conjugated synthetic peptide with Glu196 (CKNGFVMLSNE). The hybridoma cells (1672 clones) were screened for the binding ability of their producing antibodies to the recombinant EGF1-4 domains (Ile117–Glu283) of the PS K196E mutant. We measured 33 plasma samples (25 with KK and 8 with KE genotypes) by a sandwich ELISA system, in which polyclonal anti-PS and the PS K196E mutation-specific monoclonal antibodies were used as capture and detection antibodies, respectively.

Results: We obtained three hybridoma cell lines producing PS K196E mutation-specific antibodies. All 8 KE plasma samples showed significantly higher absorbance at 450 nm than 25 KK plasma samples (absorbance range: KE, 0.30–1.00; KK, 0.00–0.04). Furthermore, the KE samples derived from a warfarin-treated individual and a pregnant individual also showed significantly higher absorbance than all KK samples examined (absorbance: warfarin, 0.30; pregnancy, 0.37).

Conclusion: We developed an ELISA system for detecting a PS K196E mutant in plasma. This system will be a useful tool for the rapid identification of PS K196E carriers instead of genetic analysis.

Disclosure of Interest: None declared.

PO600-MON

Modified computer-assisted strain gauge plethysmography as a simple screening test for patients with suspected deep vein thrombosis: a cohort study

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Background: We have previously evaluated Strain Gauge Plethysmography (SGP) as a simple screening test for suspected deep vein thrombosis (DVT) against whole leg compression ultrasound (CUS). A negative SGP was highly predictive of a negative CUS and could decrease the need for whole leg CUS from 69% to 48%.

Aims: To evaluate SGP with proximal CUS to determine whether a negative SGP could similarly decrease the need for proximal CUS, which is costly.

Methods: During 13 months from September 2013 we performed SGP on a cohort of 236 patients presenting to a single community clinic with suspected DVT. We used the diagnostic algorithm recommended by the UK National Institute for Health and Clinical Excellence (NICE Clinical Guideline 144, 2012) using a 2-level Wells score and a point of care quantitative d-dimer (cardiac d-dimer). Proximal CUS was carried out as per the NICE algorithm. SGP was carried out but the results were not used to influence the diagnostic pathway. All patients discharged with a DVT excluded had a 3-month follow up to determine the incidence of DVT in this group. The follow up rate was 93% (11/236 not contactable). The primary endpoint was proximal DVT.

Results: 156 (66%) patients with a negative SGP either had a negative CUS or, if they did not have a CUS on clinical grounds, had no DVT at 3-month follow up. 69 patients had a positive SGP. 14 had DVTs on CUS and 55 had either a negative CUS (46) or no DVT at 3-month follow up (9). Sensitivity and Negative Predictive Value were both 100% (95% CI 77–100% and 96–100% respectively). DVT prevalence was 6%. A negative SGP was highly predictive for no proximal DVT, with no false negatives. The rate of CUS scanning in the study was 63%. If patients with a negative SGP had not been scanned with CUS, the CUS scanning rate would have been 31%, a decrease of 51%.

Conclusion: GP is sensitive to rule out proximal DVT and can improve the efficiency and cost-effectiveness of suspected DVT screening when proximal CUS is used in the diagnostic process.

Disclosure of Interest: None declared.

PO601-MON

Modified strain gauge plethysmography can decrease the need for compression ultrasonography in patients with suspected deep vein thrombosis: a retrospective multicentre cohort study

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Background: About 15% of patients with suspected deep vein thrombosis (DVT) have a compression ultrasound (CUS) confirmed clot. Screening with a clinical decision rule and a d-dimer (DD) can exclude a DVT in approximately 1/3rd. 2/3rds will still require a CUS that

may be normal in up to 75%, which is costly. Previous single centre studies have shown that strain gauge plethysmography (SGP) has a negative predictive value (NPV) for DVT of $\geq 97\%$ and could decrease the need for CUS. A recent single centre study by one of us (AH), using modified SGP, showed a NPV for proximal DVT of 100% and a potential reduction in CUS from 69% to 48%.

Aims: To further evaluate the efficacy of SGP in DVT screening in a multicentre cohort of patients.

Methods: A retrospective multicenter cohort study was carried out on 547 patients presenting with suspected DVT to 2 hospital and 2 community-based services. SGP was performed in addition to screening with a 2-level Wells score and a quantitative laboratory or point of care DD. CUS was carried out according to local protocols. 3-month follow up (3-MFU) was carried out on all patients discharged with a DVT excluded. The primary endpoint was proximal DVT.

Results: 377 patients with a negative SGP had either a negative CUS or no DVT at 3-MFU in patients who on clinical criteria did not have CUS (true negative). 4 patients with a negative SGP had proximal DVTs (false negative). Of 160 patients with a positive SGP, 56 had a positive CUS (true positive). 104 had either a negative CUS or no DVT at 3-MFU in those who had no CUS as above (false positive). Sensitivity was 92% (95% CI 82–98%) with a NPV of 99% (95% CI 97–99.7%). DVT prevalence was 10%. The CUS scanning rate was 73%. If patients with a negative SGP had not been scanned the scanning rate would have decreased by 59% to 30%.

Conclusion: A negative SGP is highly predictive of a negative CUS for proximal DVT and its additional use in DVT screening could significantly decrease the need for CUS in primary and secondary care.

Disclosure of Interest: None declared.

PO602-MON

Ultrasound diagnostic criteria and clot diameters of isolated distal deep venous thrombosis

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Background: Isolated distal deep vein thrombosis (IDVT) is a frequent finding in symptomatic outpatients, but the ultrasound diagnostic criteria for IDVT are still uncertain. It has been proposed that only clots ≥ 5 mm in diameter under compression may be considered as IDVT.

Aims: To assess clot characteristics and the rate of recanalization after antithrombotic treatment during the acute phase of IDVT.

Methods: In a prospective, multicenter study we enrolled 104 symptomatic outpatients (age 68 ± 14 y, male 39%) in whom a complete real-time B-mode and color Doppler ultrasonography examination revealed an IDVT. The following veins were scanned transversally over their entire length: posterior tibial veins, fibular veins, internal and external gastrocnemius veins, and soleal veins. The degree of occlusion was measured as diameter of the clot during maximal compression. Patients were treated with low molecular weight heparins for 6–8 weeks and then ultrasonography examination was repeated.

Results: Thrombosis confined only to the muscle veins was detected in 75 patients, axial veins in 23 patients, and 6 patients had both muscular and axial calf thrombosis. A total of 195 clots were found with a diameter of 5.3 ± 1.9 mm (IQR 4–6.5 mm). Only 52% of patients had a clot diameter ≥ 5 mm, whereas 98% of patients had a clot diameter ≥ 2.5 mm. Mean Clot diameters were 6.2 ± 1.9 mm for posterior

tibial veins, 4.7 ± 1.8 mm for fibular veins, 5.2 ± 1.8 mm for internal gastrocnemius veins, 4.9 ± 1.7 mm for external gastrocnemius veins, and 5.6 ± 2.2 mm for soleal veins. A complete recanalization after heparin treatment was found in 66% of patients and was not correlated with clot diameter.

Conclusion: In everyday clinical practice the criteria for IDVT diagnosis are variable being only half the patients with a clot diameter ≥ 5 mm.

Disclosure of Interest: None declared.

PO603-MON

Outcome of screening for occult malignancy in patients presenting with unprovoked venous thromboembolism at a large tertiary hospital in central London

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Background: It is thought that up to 20% of first venous thromboembolic events (VTE) are associated with an underlying cancer¹⁻⁵. The National Institute for Health and Clinical Excellence in England, Clinical Guideline 144 (NICE CG144) recommends that patients over 40 years old, presenting with the first unprovoked VTE are screened for occult malignancy⁶.

Aims: The aim of our study was to determine the incidence of malignancy after screening of patients managed in an ambulatory setting following a first unprovoked VTE.

Methods: A retrospective review of medical records of patients presenting to our service with VTE between 1st January 2013 to 31st December 2014 was performed. Patients with unprovoked VTE over 40 years old were identified and outcomes of cancer screening investigations as recommended by NICE CG144 (clinical history and examination, full blood count, renal and liver function, PSA for men, mammography in women, chest x-ray, ultrasound abdomen $\pm$ CT abdomen/pelvis as indicated) were reviewed.

Results: 239 patients with VTE were managed in our ambulatory service. Out of 189 over 40 years, 58 had unprovoked VTE (35 males, 23 females; median age 54; range 40 – 86 years). 42 had deep vein thrombosis (DVT) and 16 had pulmonary emboli (PE).

All patients with unprovoked VTE underwent cancer screening as recommended by NICE CG144. 2/58 (3.4%) patients were found to have a malignancy; both were cases of lung cancer in heavy smokers who had abnormal chest X-rays. Screening prompted additional investigations in 7 cases; 3 abdominal MRI scans, one gynaecology review, 2 colonoscopies and one breast biopsy, none of which yielded a diagnosis of malignancy.

Conclusion: Given the low yield of the extensive screening tests performed, further evidence is required to establish the utility of screening for occult malignancy in patients with unprovoked VTE. In addition, screening for malignancy may lead to further unnecessary testing; increasing cost and unnecessarily causing anxiety to patients.

Disclosure of Interest: None declared.

Venous thromboembolism – epidemiology I

PO604-MON

A prospective study of venous thromboembolism incidence and risk factors in severe sepsis and septic shock

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Background: Sepsis is characterized by dysregulated activation of the hemostatic system, promoting thrombotic events that contribute to organ failure and death. Nevertheless, prospective studies on VTE incidence and risk factors during sepsis remain absent.

Aims: We sought to determine VTE incidence, risk factors, and outcomes in septic patients.

Methods: We prospectively studied consecutively enrolled patients ($n = 113$) with severe sepsis or septic shock in three hospitals. Patients provided informed consent. IRB approval was obtained. Patients underwent comprehensive venous duplex compression ultrasonography (CUS) prior to discharge and were also followed for symptomatic deep vein thrombosis (DVT), pulmonary embolism (PE), and all-cause mortality. Multivariate regression analyses identified risk factors for VTE.

Results: The mean APACHE II score was 18.2 ± 7 , age was 50 ± 18 years, and 28-day mortality was 21%. All patients received guideline-recommended VTE prophylaxis (80.5% received chemoprophylaxis and 19.5% received mechanical prophylaxis due to a contraindication). 37.2% of patients developed VTE and the majority of events (76%) were symptomatic. 64% of VTE events were proximal DVT and/or symptomatic PE. An indwelling central venous catheter (CVC, OR 2.82, 95% CI 1.07–7.38) and the duration of mechanical ventilation (OR 1.14, 95% CI 1.00–1.29) were significantly associated with VTE. Patients with VTE had a significantly longer ICU length of stay (13.3 vs 18.5 days) and higher 28-day mortality (28.6% vs. 16.6%).

Conclusion: To our knowledge, this is the first prospective multicenter study specifically investigating VTE incidence and risk factors in septic patients. VTE occurred in almost 40% of patients despite universal, guideline-recommended prophylaxis. VTE was associated with increased ICU length of stay and mortality. Dysregulated hemostasis in sepsis may contribute to thrombosis and more effective VTE prevention strategies are needed. Early extubation and removing CVCs may prevent VTE in septic patients.

Disclosure of Interest: None declared.

PO605-MON

Venous thromboembolism in northeast Melbourne, Australia: evaluation of epidemiology, risk factors and treatment strategies in the warfarin era

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Background: Venous thromboembolism (VTE) is a major cause of morbidity and mortality. While most studies have analysed specific aspects of VTE.

Aims: We aim to provide a holistic evaluation of local VTE management in the warfarin era.

Methods: Retrospective evaluation of VTE from July 2011 to December 2012 at Austin and Northern Health, Melbourne including demographics, provoking factors, management, complications and mortality.

Results: 1029 episodes were identified including 26 recurrences – 577 (56%) pulmonary embolism (PE), 428 (42%) deep venous thrombosis (DVT). Median age was 63 years with male predominance (52%), including in the DVT subgroup (57% vs 48%, $P = 0.003$) although there was no gender difference for PE. 20% reported prior VTE. Left limb DVT was more common (49% vs 43%, $P = 0.0008$). 247 patients (24.6%) had cancer and were excluded from analysis. In non-cancer patients, 63% had provoked VTE and thrombophilia screen was performed in 41%. The median duration of anticoagulation was 6 and 7 months for DVT and PE respectively. The majority (90%) was on warfarin for long-term anticoagulation. 5% required further interventions – IVC filter ($n = 28$) and thrombolysis ($n = 15$). 38% had end-of-treatment repeat imaging and residual clot was observed in 40%. Clot persistence was associated with increased recurrence risk ($P < 0.001$). 8% had recurrent thrombosis with no difference between provoked vs. unprovoked VTE (7.5% vs 9.0%, $P = 0.45$). 5% reported grade III/IV bleeding, independent of duration of anticoagulation. Patients on Enoxaparin had higher risk of bleeding (28% vs 10%, $P < 0.001$). The mortality rate in this non-cancer cohort was 11%.

Conclusion: VTE is associated with a significant mortality rate (11%) in non-cancer patients. Risk factors for recurrence identified include residual clot on repeat imaging. Evaluation of predictive and preventive factors of new thrombosis remains a crucial challenge. This data will serve as an important baseline for future comparison in the new era of novel oral anticoagulants.

Disclosure of Interest: None declared.

PO606-MON

Low incidence of deep vein thrombosis after total knee arthroplasty in an Asian population

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Background: Deep vein thrombosis (DVT) of the lower limbs is an important complication post total knee arthroplasty (TKA). Current guidelines recommend routine chemical prophylaxis to all patients undergoing this procedure but this is rarely done in Asia as it is believed that Asians have a lower risk of VTE. However, recent evidence suggest otherwise.

Aims: We evaluated the incidence of DVT after TKA in a multi-ethnic Asian population, as well as the management and outcome of patients with post-surgery DVTs.

Methods: We conducted a retrospective study of consecutive patients who underwent TKA in our hospital from 1st January 2004 to 30th June 2012. It was mandated that all patients after TKA be put on mechanical calf pumps with a post-operative day 3 to 5 doppler ultrasound (DUS) of bilateral lower limbs. Patients were followed up for a period of 3 months.

Results: There were a total of 1885 patients (1359 Chinese, 207 Malays, 271 Indians, 28 Caucasians, 20 others). 1783 (94.6%) patients has DUS of the lower limbs with 144 diagnosed with DVT, giving an incidence of 8.1%. There were 23 (16.0%) proximal DVTs and 121 (84.0%) distal DVTs. There were 12 (8.5%) symptomatic DVTs. 46 (31.9%) patients were anti-coagulated (14 proximal, 32 distal DVTs) with no major bleeding complications. In non-treated patients, none

had clot progression. After 3 months of follow up, no additional DVTs occurred. 4 patients had pulmonary embolism (PE) and 2 patients died (1 DVT, 1 PE), giving an overall mortality of 0.1%. Length of operation and hospital stay were not associated with an increased risk of DVT.

Conclusion: In one of the largest studies specifically investigating the incidence of DVT after TKA, we found that the incidence is low at 8.1%. This is in contrast to recent studies that showed post-operative VTE rates similar to Western populations. We believe that our routine use of mechanical prophylaxis post-operatively may have helped reduced the DVT rates.

Disclosure of Interest: None declared.

PO607-MON

Asymptomatic thrombosis following the use of central venous lines in children

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Background: Over 50% of thromboses in children are related to central venous lines (CVLs). The rate of complications, such as Post Thrombotic Syndrome (PTS), from asymptomatic CVL-related thrombosis is unknown and hence the importance of treating children with anticoagulation to prevent these sequelae is unclear.

Aims: This study will determine the frequency and clinical outcome of asymptomatic CVL-related thrombosis in children and determine the frequency and severity of PTS following CVL placement in children.

Methods: A prospective cohort study was conducted. 205 children admitted to a paediatric intensive care unit (PICU) requiring a CVL in the jugular or femoral veins participated. The study was approved by the hospital human research ethics committee and informed consent was obtained from all parents/guardians for their child's participation. Each child had a (blinded) ultrasound of the blood vessel in which the CVL was placed during their admission. Clinical data and plasma were collected to determine risk factors for thrombosis and markers of thrombotic activity (Phase I). A second ultrasound and a PTS assessment will be performed approximately 24 months following CVL placement (Phase II).

Results: Recruitment is complete and data collection and analysis of phase I data are underway. Of 379 families approached, 205 patients consented for the study (54%). 48% of children were aged less than 1 year, with 80% of children having a cardiac diagnosis. Ultrasound imaging from 149 patients confirmed the incidence of CVL-related thrombosis was 22.1% ($n = 33$). Only one patient was symptomatic.

Conclusion: This study will determine both the incidence of symptomatic and asymptomatic CVL-related thrombosis in children in PICU and the incidence and severity of PTS. Additionally, this study will determine if there is or is not clinical value in performing routine ultrasound screening of all children with a CVL for the presence of thrombosis.

Disclosure of Interest: None declared.

PO608-MON

Does diabetes influence outcome within three months after venous thromboembolism? Findings from 4011 cases enrolled in the worldwide RIETE registry

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Background: Increasing evidence of concomitant hypercoagulability suggests an association between diabetes and the risk of venous thromboembolism (VTE), however results of epidemiological studies are conflicting. In addition, VTE shares many risk factors with atherosclerotic cardiovascular diseases, including diabetes.

Aims: To evaluate the outcome of patients with and without diabetes, after a VTE episode.

Methods: RIETE is an ongoing, worldwide, prospective cohort of patients presenting with symptomatic VTE objectively confirmed, and followed-up for at least 3 months. We used propensity scoring to create a match sample (diabetes and no-diabetes patients) for the major clinical events within 3 months from VTE.

Results: A population of 26,484 patients was considered for the analysis; 4011 (15.1%) had diabetes. Patients with diabetes were significantly older (73 ± 12 vs 63 ± 19 years, $P < 0.001$), and with a greater burden of comorbidity and concomitant risk factors for vascular disease (obesity, hypertension). Thromboprophylaxis had been used in 36.9% and 37.0% of diabetes and no-diabetes patients. At 3-month follow-up, a slight higher percentage of patients with diabetes had recurrent VTE (1.6% vs 1.3%), major bleeding (2.1% vs 1.5%) or arterial events (1.1% vs 0.4%, $P < 0.001$). According to propensity score matching analysis, diabetes patients had a significantly higher occurrence of arterial ischemia (1.2% vs 0.6%, OR 1.88 [1.27–2.76], $P = 0.001$) and of the composite end-point recurrent VTE + major bleeding + arterial events (4.7% vs 3.5%, OR 1.35 [1.12–1.62], $P = 0.002$).

Conclusion: Diabetic patients seem to have a slightly worse prognosis within 3 months after VTE, in particular they had an increased occurrence of arterial events (though of limited absolute rate). This could support the hypothesis of a 'domino' effect between venous and arterial events, and leads us to think about some therapeutic attitudes following a VTE episode (i.e. interruption of antiplatelet therapy).

Disclosure of Interest: None declared.

PO609-MON

Time trends in incidence rates of venous thromboembolism in a large cohort recruited from the general population

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Background: Alterations of risk factor levels, prevention strategies, diagnostic tools and treatment modalities over time may alter the rate of venous thromboembolism (VTE) in a community. Changes in the incidence of VTE during the last 15 years have not been extensively studied.

Aims: To study time trends in the incidence rates of deep vein thrombosis (DVT) and pulmonary embolism (PE) in a large population-based cohort.

Methods: A total of 26855 subjects, aged 25–97 years, were enrolled in the Tromsø study in 1994/95 and followed up to December 31st 2012. All symptomatic, objectively confirmed, incident VTEs were identified using multiple sources (hospital discharge-, radiology procedure- and autopsy registry) and carefully validated by review of medical records. Age-adjusted biennial incidence rates (IR) with 95% confidence interval (CI) were calculated using direct standardization with the age-distribution in 2004 as the reference population. The study was approved by the regional ethical committee and all subjects gave their informed written consent.

Results: Between January 1st 1996 and December 31st 2012, 693 VTEs occurred during a total of 368150 person-years (PY) of follow-up. The incidence rate of VTE increased from 1.5 (95% CI 1.1–1.9) per 1000 PY in 1996/97 to 1.9 (95% CI 1.3–2.4) per 1000 PY in 2012. There was a marked increase in the rates of PE ranging from 0.4 (95% CI 0.2–0.6) in 1996/97 to 1.0 (95% CI 0.6–1.4) per 1000 PY in 2012, whereas the DVT rates remained relatively constant (1.1, 95% CI 0.7–1.4 in 1996/97 and 0.8, 95% CI 0.4–1.2 per 1000 PY in 2012).

Conclusion: Despite advances in prophylaxis, the incidence rate of VTE has increased slightly during the last 15 years, mainly due to an increase in PE. Although the introduction of better diagnostic tools to some extent may explain the increase in PE, our findings suggest that there is still a need for improvement in risk factor management and prevention strategies of first-time VTE.

Disclosure of Interest: None declared.

PO610-MON

Poor self-rated health is associated with future risk of venous thromboembolism in women. The Tromsø study

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Background: Poor self-rated health status is associated with arterial cardiovascular outcomes and all cause-mortality. The relation between self-rated health and risk of venous thromboembolism (VTE) has never been studied.

Aims: To investigate the association between self-rated health and future risk of VTE in men and women recruited from a general population.

Methods: Men ($n = 12726$) and women ($n = 14089$) enrolled in the Tromsø study in 1994/95 were asked to report their current health

status ('Poor', 'Not so good', 'Good' and 'Very good'). All first VTE events among the participants were validated and recorded up to Dec 31, 2012. Cox-regression models with age as time scale were used to calculate hazard ratios (HR) of VTE according to health status adjusted for body mass index, smoking, physical activity, education level, diabetes, and history of cardiovascular diseases or cancer.

Results: A total of 709 VTE events occurred during a median of 17.7 years of follow-up. There was no association between self-rated health status and VTE in men (P for trend across categories=0.4). Contrary, the risk of VTE increased across categories of worse health in women ($P < 0.0001$). Women who reported poor health had almost 3-fold higher risk of VTE than women who reported very good health (HR: 2.93, 95% CI: 1.57–5.49). Similar risk estimates were found for provoked and unprovoked VTE in women, and cause-specific analyses revealed that the association was not mediated by development of cancer, myocardial infarction or stroke during follow-up (cause-specific HR for 'Poor' vs. 'Very good': 3.22, 95% CI: 1.44–7.18).

Conclusion: Our findings indicate a sex-specific impact of self-rated health on VTE risk. Women with poor health, but not men, had increased risk of future VTE even after adjustment for important confounders and co-morbidities. The mechanism behind this association remains unclear.

Disclosure of Interest: None declared.

PO611-MON

Oral and inhaled corticosteroid use has a time-dependent association with recurrent pulmonary embolism

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Background: Chronic inflammatory diseases and pulmonary diseases such as asthma and COPD predispose to the development of venous thromboembolism. Both inhaled and oral corticosteroids are used as treatment for asthma and COPD. Previous studies have shown that patients who use inhaled or oral corticosteroids have an increased risk of a first venous thromboembolism. It is not known whether they are also at increased risk for recurrent events.

Aims: To analyse the association between oral and/or inhaled corticosteroid use and the risk of recurrent pulmonary embolism (PE).

Methods: A nested case-control study was performed using the PHARMO Record Linkage System, which combines drug prescription data (ATC codes) and hospital admission and discharge codes (ICD-9 codes). All patients had a primary diagnosis of PE and a prescription for vitamin K antagonists and had a median follow-up of 14 months. Patients with a diagnosis of recurrent PE were matched to controls without recurrent PE. Conditional logistic regression analysis was used.

Results: We analysed 384 cases of recurrent PE and 1030 controls. There was a clear time-dependent association between corticosteroid use and the risk of recurrent PE. Patients using oral corticosteroids in the month prior to the event showed an increased risk (odds ratio (OR) 3.74; 95%CI 2.04–6.87; $P < 0.01$) of recurrent PE, which normalised for use in the prior 1–6 months ($P = 0.82$), to even a protected effect when oral corticosteroids were used >6 months prior to the event (OR 0.46; 95%CI 0.28–0.74; $P < 0.01$). The same pattern was observed for inhaled corticoster-

oids, although less strong (<1 month OR 1.55; 95%CI 0.90–2.67; $P = 0.12$ and >6 months OR 0.52; 95%CI 0.30–0.90; $P = 0.02$). Combination of inhaled and oral corticosteroids increased recurrent PE risk for use in the month prior to the event (OR 4.60; 95%CI 1.54–13.76; $P < 0.01$).

Conclusion: Oral and inhaled corticosteroid use is associated with a time-dependent association with recurrent PE.

Disclosure of Interest: None declared.

PO612-MON

Case fatality and recurrence rates after first venous thromboembolism in a large population-based cohort

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Background: Previous reports on recurrence and mortality rates after a first episode of venous thromboembolism (VTE) vary considerably, and most of the studies were conducted several decades ago. Advances in management and treatment of venous thrombosis during the last 15 years may have influenced the rates of clinical outcomes.

Aims: To estimate the rates of recurrence and mortality after a first VTE event in patients recruited from a large population-based cohort.

Methods: From the Tromsø study, patients ($n = 710$) with a first, symptomatic, objectively confirmed VTE were included and followed in the period 1994–2012. Recurrent episodes of VTE were identified using multiple sources (hospital discharge-, radiology procedure- and autopsy registry) and carefully validated by review of medical records. Information of deaths was obtained from the National Population Registry of Norway. Incidence rates (IR) with 95% confidence interval (CI) of VTE recurrence and mortality after the first year of VTE and the remaining follow-up period were calculated. The study was approved by the regional ethical committee and all subjects gave their informed written consent.

Results: The mean age of the patients was 68 (range 28–102) years and 166 (23.4%) had active cancer at the time of the first VTE. There were 114 VTE recurrences and 333 died during a median of 7.7 (range 0.04–18.2) years of follow-up. The one-year recurrence rate was 7.8% (95% CI 5.8–10.6) whereas the recurrence rate in the remaining follow-up period (1–18 years) was 3.0 (95% CI 2.3–3.8) per 100 person-years (PY). The risk of dying was highest in the first year following the VTE. The overall 1-year case fatality rate was 29.9 (95% CI 25.7–34.8) per 100 PY, and in those without active cancer the corresponding rate was 14.7 (95% CI 11.6–18.5) per 100 PY.

Conclusion: Despite advances in VTE management, rates of adverse events remained fairly high especially in the first year following a VTE.

Disclosure of Interest: None declared.

PO613-MON

Time trends and case fatality rate of pulmonary embolism during 11 years of observation in northwestern ItalyDentali F¹, Ageno W¹, Pomero F², Fenoglio L², Squizzato A³ and Bonzini M¹¹Clinical Medicine, Insubria university, Varese; ²Clinical Medicine, Cuneo Hospital, Cuneo; ³Clinical Medicine, Insubria University – Varese, Varese, Italy**Background:** Pulmonary embolism (PE) is a common and increasingly diagnosed disorder with high mortality and morbidity rates. However, population-based information on its incidence and prognosis remains limited.**Aims:** To better assess the incidence and the short term prognosis of PE on a large population during a long period of observation.**Methods:** We conducted a large epidemiology study collecting data on hospitalization for PE (from 2002 to 2012) in a population of about 13 millions people in Northwestern Italy. Patients were identified using the ICD-9-CM codes: 415.11, 415.19; gender and age specific incidence rate of PE during the study period were estimated using the resident population for each year of the study. Furthermore, time trends in the in-hospital PE-related mortality and case fatality rate were calculated. Results were adjusted for possible confounders.**Results:** 60,853 patients with a first event of PE were included in the analysis. The overall crude incidence rate for the entire study period was 55.4 and 40.6 events per year per 100,000 inhabitants for women and men, respectively ($P < 0.001$). This difference completely disappeared when the incidence rates for the two populations were standardized according to age. The incidence of PE significantly increased in both genders during the study period. In-hospital case fatality rate significantly decreased throughout the study period ($P < 0.001$) in women (from 15.6% to 10.2%) and in men (from 17.6% to 10.1%). The observed decrease of the in-hospital case-fatality throughout the study period remained significant also after adjustment for possible confounders.**Conclusion:** Time trends over an 11-year period show an increasing incidence of PE, but a significant reduction in mortality during hospitalization. Reduction in the case fatality rate remained significant after adjustment for possible confounders**Disclosure of Interest:** F. Dentali Grant/Research Support from: Bayer, BMS/Pfizer, Boehringer, Consultant for: bayer, W. Ageno: None Declared, F. Pomero: None Declared, L. Fenoglio: None Declared, A. Squizzato: None Declared, M. Bonzini: None Declared.

PO614-MON

Natural history and antithrombotic treatment of incidentally detected splanchnic vein thrombosis: findings from the ISTH registryRiva N¹, Ageno W¹, Schulman S², Beyer-Westendorf J³, Bang S-M⁴, Pasca S⁵, Poli D⁶, Verhamme P⁷, Alatri A⁸, Colaizzo D⁹, Rancan E¹, Rezende SM¹⁰, Piana A¹¹, Bazzan M¹², De Sancho MT¹³, Ciantar E¹⁴, Elli E¹⁵, Zawilska K¹⁶, Dentali F¹ and IRSVT study investigators¹¹Department of Clinical and Experimental Medicine, University Of Insubria, Varese, Italy; ²Department of Medicine, McMaster University, Hamilton, Ontario, Canada; ³Center for Vascular Medicine and Department of Medicine III, Division of Angiology, University Hospital 'Carl Gustav Carus', Dresden, Germany; ⁴Department of Internal Medicine, Seoul National University, Seoul, Korea; ⁵Center for Hemorrhagic and Thrombotic Diseases, University Hospital, Udine; ⁶Thrombosis Center, Careggi Hospital, Florence, Italy; ⁷Leuven University, Leuven, Belgium; ⁸Centro Emostasi e Trombosi, A.O. Istituti Ospitalieri, Cremona; ⁹IRCCS Casa Sollievo della Sofferenza, S. Giovanni Rotondo, Italy; ¹⁰Departamento de Clínica Médica, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; ¹¹Department of Internal Medicine, University of Genoa, Genoa; ¹²UOSD Ematologia e Malattie Trombotiche, Ospedale San Giovanni Bosco, Torino, Italy; ¹³Hematology-Medical Oncology Division, Weill-Cornell Medical College, New York, USA; ¹⁴Leeds General Infirmary, Leeds, UK; ¹⁵Hematology Division, S. Gerardo Hospital, Monza, Italy; ¹⁶Department of Hematology, J. Strus Hospital, Poznan, Poland**Background:** Splanchnic vein thrombosis (SVT) is often diagnosed incidentally. There is very little information on the natural history of patients with incidentally detected SVT (IDSVT) and therapeutic management remains controversial.**Aims:** To evaluate the antithrombotic treatment and the outcomes of IDSVT in real-life clinical practice.**Methods:** International prospective registry of consecutive SVT patients, enrolled from 2008 to 2012. Risk factors, therapeutic strategies and outcomes were analysed separately for patients with IDSVT. Clinical outcomes during the 2-year follow-up were reviewed by a Central Adjudication Committee and classified as vascular events (venous or arterial thrombosis) and major bleeding (ISTH definition plus the need for hospitalization).**Results:** 177 of 604 patients, enrolled from 31 centers, had IDSVT (median age 57 years, 66.7% males, 78.0% portal vein thrombosis). Provoking factors included liver cirrhosis (46.3%) and solid cancer (35.0%); IDSVT was unprovoked in 15.3%. Sixty-eight patients (38.4%) were not anticoagulated; 57 (32.2%) received parenteral anticoagulants alone (median duration 6 months, IQR 5–12.2) and 52 (29.4%) were started on vitamin K antagonists (median 24 months, IQR 12–24). Median follow-up duration was 2 years (IQR 1–2); 6 patients (3.4%) were lost to follow-up. The overall incidence of recurrent thrombotic events was 8.0/100 patient-years (pt-y) (95%CI 5.2–12.1) and of major bleeding 3.3/100 pt-y (95%CI 1.7–6.3). The incidence in never-treated patients was 11.5/100 pt-y and 2.3/100 pt-y, respectively. At multivariate analysis, anticoagulant treatment was associated with lower rates of vascular events (HR 0.84, 95%CI 0.74–0.94) without increasing the risk of major bleeding.**Conclusion:** IDSVT is not uncommon and is frequently associated with cirrhosis and solid cancer. In real-world, approximately two thirds of these patients receive anticoagulant treatment. The rate of vascular complications is relevant and anticoagulant treatment appears to be safe and effective.**Disclosure of Interest:** N. Riva: None Declared, W. Ageno: None Declared, S. Schulman: None Declared, J. Beyer-Westendorf: None Declared, S.-M. Bang: None Declared, S. Pasca: None Declared, D.

Poli: None Declared, P. Verhamme: None Declared, A. Alatri: None Declared, D. Colaizzo: None Declared, E. Rancan: None Declared, S. M. Rezende: None Declared, A. Piana: None Declared, M. Bazzan: None Declared, M. T. De Sancho: None Declared, E. Ciantar: None Declared, E. Elli: None Declared, K. Zawilska: None Declared, F. Dentali: None Declared, IRSVT study investigators Grant/Research Support from: This study was supported by a grant from Pfizer, Canada, to ISTH. The authors have no relevant conflicts to declare in relation to this abstract.

PO615-MON

Deep vein thrombosis in septic patients in the intensive care unit

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Background: Critically ill patients are at high risk for developing deep vein thrombosis (DVT). Severe sepsis and septic shock is a subgroup of critically ill patients at risk for DVT. There are limited data on the incidence and risk factors of DVT in this patient subgroup.

Aims: To report the incidence of new DVT and investigate risk factors in critically ill septic patients.

Methods: A retrospective cohort study was performed to assess the incidence and risk factors of DVT in septic patients. All consecutive patients with sepsis, admitted from November 2011 through October 2013 directly in our Intensive Care Unit were analyzed. All included patients were >18 years old and had a venous duplex within 60 days of initial ICU admission. Patients on therapeutic anticoagulation or known DVT were excluded. We employed bivariate analysis to assess the association between various risk factors and the presence DVT.

Results: 127 patients met the selection criteria and 18 (14.2%) had DVT. The mean age was 70.72 ± 15.16 years (range 29 – 95) and 45.7% were males. The mean length of ICU stay was 6.35 ± 7.83 days. Notable findings were the non-significant association of APACHE II and SAPS II scores, pharmacologic DVT prophylaxis, vasopressor requirements and mechanical ventilation with positive cases ($P = 0.916$, $P = 0.920$, $P = 0.746$, 0.801 and 0.737 respectively). Other comorbidities (Congestive Heart Failure, End-Stage Renal Disease, Diabetes, Hypertension and COPD) did not show any significant correlation with DVT cases. In contrast, there was significantly positive correlation of concurrent malignancy with DVT ($P = 0.036$).

Conclusion: Our study shows that a significant proportion of critically ill septic patients have DVT, with active malignancy being a risk factor. High clinical suspicion should be engaged in these patients, since DVTs are clinically silent and risk factors fail to predict them. Future large studies are necessary to determine independent predictors and the role of screening for DVT in this patient subpopulation.

Disclosure of Interest: None declared.

PO616-MON

Factor V is associated with a moderately increased risk of venous thrombosis

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Background: Altered blood coagulation factor levels are well-known risk factors for venous thrombosis (VT). The procoagulant factor V (FV) serves as a cofactor for the factor Xa-dependent activation of prothrombin. Conversely, FV also functions as an anticoagulant by enhancing activated protein C-mediated inactivation of activated factor VIII (FVIII). This dual role of FV complicates conclusions on the contribution of FV to VT occurrence, and in previous studies both high and low FV levels have been associated with VT risk.

Aims: Here we elucidated the association of FV with VT using a large case-control study and we adjusted the observed risk associations for FVIII levels, a strong risk factor for VT, to study possible mediation through FVIII.

Methods: The FV and FVIII antigen levels of 2379 cases and 2943 controls from the population-based case-control MEGA study were measured, on the basis of which the participants were categorized according to the 1st, 2.5th 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentile of factor levels. Odds ratios (ORs) were calculated using logistic regression and adjusted for age and sex and FVIII, when applicable.

Results: Median FV levels were slightly higher in patients than in controls (0.94 vs. 0.92 U mL⁻¹). Age and sex adjusted ORs increased with rising FV levels, with the highest OR of 1.86 (95%CI 1.46 – 2.37) for the 95th percentile as compared to the reference group (25th–50th percentile). The OR of the lowest group (<1st percentile) was slightly elevated (OR 1.46 ; 95%CI 0.87 – 2.43). FVIII adjustment led to attenuation of the ORs, except for the lowest group, which maintained an increased risk (OR 1.68 ; 95%CI 0.97 – 2.91).

Conclusion: In conclusion, we observed an association between high FV levels and VT. This effect disappeared after adjustment for FVIII, which indicates that the risk is mainly mediated by FVIII. The association between low levels and risk was not affected by FVIII levels. Our data indicate that FV levels are weakly associated with an increased risk of VT.

Disclosure of Interest: None declared.

PO617-MON

Thrombin generation and the risk of venous thrombosis: results from a large population-based case-control study

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Background: Thrombin generation (TG) is a global coagulation test that can be utilized as an intermediate phenotype to detect prothrombotic states leading to venous thrombosis. Previous studies using various TG protocols have shown associations between increased TG and venous thrombosis. However, optimal experimental TG conditions to predict venous thrombosis are yet to be defined.

Aims: To estimate the risk of venous thrombosis associated with TG parameters measured at various TG assay conditions.

Methods: We measured TG at various conditions in the MEGA study, a large population based, case control study on the etiology of venous thrombosis. Cases enrolled in the study were diagnosed with a first episode of venous thrombosis and aged between 18 and 65 years old. TG was measured at low (2 pM) and high (10 pM) tissue factor (TF) concentrations, in the absence or presence of activated protein C (5 nM). Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated after adjustment for age and sex.

Results: Plasma samples for TG were available in 2364 cases and 2938 controls. Individuals on oral anticoagulant treatment ($n = 315$) were excluded from analyses. Under all experimental conditions thrombin generation was increased in cases with venous thrombosis compared with controls. From the TG parameters, endogenous thrombin potential and peak height were dose-dependently associated with venous thrombosis under all conditions. No such trend could be observed for the lag time. Risk estimates were highest when a low TF trigger concentration was used, i.e., for peak height: adjusted OR Q4 vs Q1 = 6.3 (95% CI 5.2–7.6) and for ETP: adjusted OR Q4 vs Q1 = 5.6 (95% CI 4.6–6.8).

Conclusion: Increased TG peak height and ETP are associated with an increased risk of venous thrombosis under all conditions. However, the risks were highest when a low TF trigger concentration is used.

Disclosure of Interest: None declared.

Venous thromboembolism – therapy I

PO618-MON

Two weeks of low molecular weight heparin for isolated symptomatic distal vein thrombosis (twister study): interim analysis of first 100 patients

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Background: Most deep vein thrombi (DVT) originate from a small calf vein (distal vein) thrombus, however 80–90% of distal DVT lyse spontaneously and are probably of minimal clinical consequence. The treatment and management of isolated distal DVT (ID-DVT) thrombi is controversial, with treatments ranging from withholding of anticoagulation and a repeat compression vein ultrasound (CUS) of the leg veins in a week, to three months of full dose anticoagulation.

Aims: The TWISTER Study is a multicentre Australasian study examining the safety of two weeks of anticoagulation for a first episode of symptomatic ID-DVT. The primary outcome is symptomatic recurrence of venous thrombosis DVT and pulmonary embolism, PE) within 3 months.

Methods: Patients with confirmed symptomatic distal DVT received 2 weeks of therapeutic anticoagulation (enoxaparin or rivaroxaban) and had a repeat CUS at the end of 2 weeks. If the patient was asymptomatic and there was no proximal DVT extension on CUS, treatment was stopped. If the patient was still symptomatic then treatment was continued for a further four weeks. All study events were centrally adjudicated (blinded).

Results: An interim analysis of the first 100 patients was performed. Ninety-three received enoxaparin; 7 received rivaroxaban. The duration of anticoagulation was 2 weeks (± 1 week) for 77 patients, 6 weeks for 21 patients (with 2 receiving an additional 6 weeks at the investigator's discretion), and 3 months for 2 patients due to asymptomatic proximal extension on the 2 week CUS. There were also 7 extensions within the distal venous system, with all occurring whilst on full dose anticoagulation. There has been one VTE recurrence within 3 months (1.3%; 95%CI 0–3.8%), a distal DVT in the contralateral leg.

Conclusion: Our preliminary data suggest two weeks of therapeutic anticoagulation is sufficient for the majority of patients (77%) with a first episode of symptomatic ID-DVT, with a low rate of VTE recurrence within 3 months (1.3%).

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PO619-MON

A Canadian consensus on venous thromboembolism (VTE) prophylaxis prevention in thoracic surgery, a national Delphi consensus survey

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Background: The incidence of Venous Thromboembolic Events (VTE) after resection of thoracic malignancies is reported to be as high as 15%, but the evidence required to support prophylaxis guidelines does not exist.

Aims: We conducted a modified Delphi consensus process to survey Thoracic Surgeons, Thoracic Anesthesiologists and Thrombosis experts across Canada. The questions addressed perioperative risk factors for VTE, impact of those factors on selecting extended prophylaxis, type of preferred prophylaxis (medical and/or mechanical), timing of treatment initiation and duration.

Methods: Three survey rounds were conducted. Participants were asked to rate each parameter on a ten-point scale. Consensus was defined a priori as an item demonstrating a coefficient of variation of $\leq 30\%$ (0.3), and the item was then discontinued from future rounds.

Results: A total of 72, 57 and 50 responders participated in the rounds respectively, with a good distribution between specialties. Consensus was reached on risk factors such as previous VTE, age, cancer diagnosis, thrombophilia, poor mobilization, extended resections, and pre-operative chemotherapy. Consensus on risk factors impacting extended prophylaxis decisions was achieved on cancer diagnosis, obesity, previous VTE and poor mobilization. Almost no consensus was reached on the approach to perioperative prophylaxis, with only daily LMWH usage reaching consensus. No consensus was achieved regarding the role of mechanical prophylaxis, unfractionated heparin or timing of initiation of peri-operative treatment. VTE prophylaxis until discharge reached consensus. There was no consensus concerning extended prophylaxis.

Conclusion: There is no standard consensus on VTE prophylaxis after Thoracic Surgery in Canada. While there is a consensus between clinicians treating patients with thoracic malignancies regarding risk factors for VTE events, there is no agreement on timing of initiation of

treatment, agents used and factors potentially mandating extended duration of VTE prophylaxis.

Disclosure of Interest: None declared.

PO620-MON

Adherence to international guidelines in treatment of thromboembolic events in cancer patients: a hospital-based cohort study in Paris, France

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Background: LMWHs are recommended by international guidelines for a period of 3–6 months in patients with cancer associated thromboembolism

Aims: To assess the implementation in daily clinical practice.

Methods: Using the PMSI (prospective payment system) databases, all hospitalisations from January to December 2012, in three MCO (medicine, surgery, obstetrical) hospitals (Assistance Publique Hôpitaux de Paris) were retrospectively analyzed to look for patients with the reported diagnosis of cancer and venous thromboembolism (VTE) (as a primary or secondary diagnosis). Compliance with guidelines was assessed by comparing the treatment received by the patient at the time of hospitalization to guidelines. T1 was defined as the first ten days after VTE diagnosis, T2 the period from 10 days to 3 months, T3 the period between 3 and 6 months, and T4 after 6 months.

Results: 219 therapeutic strategies were assessed among 240 included patients. Adhesion to guidelines was 56% among patients during T1, but decreased during T2 and T3 (33% and 11% respectively). In T4, 65% of patients had an appropriate treatment due to less restrictive recommendations after 6 months. The overall compliance was estimated at 52% for all periods. Patients with special conditions (renal failure, thrombocytopenia, VTE recurrence on anticoagulation) had less often a treatment in accordance with recommendations (46%). Overall, 60% of all cancers were at a metastatic stage, and patients with an advanced cancer had more often a treatment in accordance with guidelines (58%). Patients who experienced pulmonary embolism were more often adequately treated (60%) than patients with deep venous thrombosis (40%)

Conclusion: Adhesion to guidelines for VTE treatment in cancer is low, with only half patients with an adequate treatment. Compliance dropped significantly after the first 10 days of treatment. Only the cancer stage seems to impact physician's prescription. Our study underlies the need to further promote adequate therapeutic strategies in usual care

Disclosure of Interest: None declared.

PO621-MON

A first-in-human, single ascending dose study of DS-1040, an inhibitor of the activated form of thrombin-activatable fibrinolysis inhibitor (TAFIa), in healthy subjects

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Background: DS-1040 is a TAFIa inhibitor under development for the treatment of thromboembolic diseases.

Aims: The objective of this first-in-human study was to assess the safety, tolerability, pharmacokinetics, and pharmacologic activity of DS-1040 in healthy subjects.

Methods: A total of 103 healthy males and females were enrolled in this double-blind, placebo-controlled, randomized, 3-part, single ascending dose study. In the first part of study, 64 young subjects, age 18–45, were randomized to receive single doses of DS-1040 (0.1 mg to 12 mg administered as 0.5 h intravenous [IV] infusions) or placebo in cohorts of 8 subjects (6 active/2 placebo per cohort). In the second part, 15 elderly subjects, age 65–75, were randomized to receive single doses of DS-1040 (3 mg and 6 mg administered as 0.5 h IV infusion) or placebo. In the third part, 24 young subjects were randomized to receive single doses of DS-1040 (10 mg to 40 mg administered as 24 h continuous IV infusions) or placebo.

Results: Overall, all doses of DS-1040 were safe and well tolerated in young and elderly subjects. Plasma exposure of DS-1040 increased proportionally with increases in dose. The terminal half-life ranged from 16.5 h to 16.8 h in young and 33.9 h to 40.7 h in elderly subjects, respectively. Elimination was primarily renal. DS-1040 administration resulted in a dose-dependent decrease of TAFIa activity and 50% clot lysis time. Increase in D-dimer levels was also observed but without clear dose dependency. No effects were observed on coagulation parameters (PT, PTT, and fibrinogen) or platelet aggregation.

Conclusion: Single doses of DS-1040 up to 40 mg given as IV infusion were safe and well tolerated in healthy subjects and demonstrated pharmacologic activity. These results support future clinical development.

Disclosure of Interest: J. Zhou Employee of: Daiichi Sankyo Pharma Development, C. Zamora Employee of: Worldwide Clinical Trials, G. Atiee Employee of: Worldwide Clinical Trials, V. Warren Employee of: Daiichi Sankyo Pharma Development, O. Yin Employee of: Daiichi Sankyo Pharma Development, J. Kochan Employee of: Daiichi Sankyo Pharma Development, J. Pav Employee of: Daiichi Sankyo Pharma Development, Y. Orihashi Employee of: Daiichi Sankyo Development Ltd., V. Vashi Employee of: Daiichi Sankyo Pharma Development, V. Dishy Employee of: Daiichi Sankyo Pharma Development.

PO622-MON

A retrospective medication use evaluation of antithrombotic therapy after total hip arthroplasty or total knee arthroplasty at university of colorado health

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Background: Patients undergoing total hip and total knee arthroplasty (THA or TKA) are at high risk of developing hospital acquired venous thromboembolism (VTE). Currently there is a debate on the relative

merits of aspirin compared to anticoagulants among clinicians. Recently standardized practices for VTE prevention in THA/TKA patients at the University of Colorado Hospital (UCH) changed from low molecular weight heparin (LMWH) to ASA providing two cohorts of patients to assess comparative effects.

Aims: (1) To assess the relative efficacy and safety of ASA compared to LMWH when used for VTE prevention post THA and TKA; (2) To identify additional patient characteristics which may help predict bleeding or thrombotic events.

Methods: Patients were identified who underwent elective THA and TKA at UCH from January 1 – April 1, 2013 (cohort #1), and from January 1 – April 1 2014 (cohort #2). Patients in cohort #1 received a LMWH strategy for VTE prevention, and those in cohort #2 received an ASA based strategy. Relevant patient characteristics and clinical data were collected up to 6 weeks post surgery. The primary endpoint was the percentage of patients in each group who developed a clinical VTE event. Secondary endpoints included the incidence of major bleeding (ISTH definition) as well as transfusion requirements between groups.

Results: 138 TKA or THA patients received LMWH, and 90 received ASA as the primary VTE prevention strategy. The primary outcome occurred in 2.9% of patients receiving LMWH, and 8.9% of patients receiving ASA ($P = 0.047$). The rates of bleeding did not differ between the LMWH and ASA groups (2.9% vs 3.3%), but the rates of transfusion was significantly higher in the LMWH group (15.3% vs 2.2%, $P = 0.0012$).

Conclusion: In our study comparing a pre and post cohort of patients receiving different VTE prevention strategies, patients on ASA had a higher rate of clinical VTE but lower rates of transfusions. Full results will be presented.

Disclosure of Interest: None declared.

PO623-MON

Platelet reactivity in patients with a history of venous thrombosis who use rosuvastatin: randomized clinical trial

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Background: Experimental studies indicate that platelets play a role in the pathogenesis of venous thrombosis and that platelet hyperaggregability increases the risk of venous thrombosis. Non-randomized studies suggest that statins exert inhibitory effects on platelets, which may explain why statin use is associated with a decreased risk of venous thrombosis.

Aims: To determine the effect of rosuvastatin on platelet reactivity in patients with a history of venous thrombosis.

Methods: Data of the ‘statins reduce thrombophilia’ (START) trial were used (registered at NCT01613794). START is a multicenter, open label randomized clinical trial, to investigate the effect of rosuvastatin (20 mg daily) on the coagulation profile of patients with a history of venous thrombosis. Platelet reactivity was measured at baseline and after 1 month in 50 consecutive patients (25 receiving rosuvastatin for 30 days and 25 without intervention). Platelet reactivity in aspirin reaction units (ARU) was measured using the VerifyNow System. The local ethics committee approved the study and all patients provided informed consent.

Results: Out of 50 consecutive participants 47 had 2 ARU measurements. Mean age at time of thrombosis was 58 years (range 21–80), most were men ($n = 35$; 75%), 13 (28%) had pulmonary embolism, and 22 (47%) patients had an unprovoked event. Mean ARU in rosu-

vastatin users was 609 at baseline and 613 at end of follow-up (mean ARU difference 5; 95%CI, –18 to 27). Mean ARU in non-rosuvastatin users was 620 at baseline and 618 at end of follow-up (mean ARU difference –2; 95%CI, –15 to 12). The mean difference in ARU change between rosuvastatin and non-rosuvastatin users was 6 (95% CI, –20 to 33). Excluding patients who used antiplatelet medication ($n = 2$), had thrombocytopenia ($n = 1$) or were strong outliers ($n = 1$), revealed similar results, i.e. no apparent effect of rosuvastatin on ARU.

Conclusion: Rosuvastatin does not affect platelet reactivity in patients with a history of venous thrombosis as measured by the VerifyNow.

Disclosure of Interest: None declared.

PO624-MON

Long-term use of low-molecular-weight heparins (LMWH) for the treatment of cancer-associated thrombosis (CAT): factors associated with non-compliance with treatment recommendations (NCTR) in clinical practice

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Background: Long-term treatment with LMWH for at least 3 months is the standard therapy for CAT patients [1]. Results from the observational TROPIQUE study have shown that 44.7% of the 409 patients with CAT were not treated according to established recommendations [2].

Aims: To identify factors associated with NCTR in the TROPIQUE patient population.

Methods: NCTR defined as: treatment duration <3 months, prescription of non-recommended LMWH, non-compliance with recommended dose (NCRD), incorrect injection schedule or use in patients with severe renal failure. A total of 22 factors related to patient's characteristics, malignancy and LMWH prescription were considered for univariate analyses. Factors significant (at 10% level) in univariate analyses were included in multivariate analyses reported here (OR [95% CI] p value).

Results: Recent surgery preceding VTE (OR: 2.1[1.3–3.6] $P = 0.005$) and bleeding within a month of VTE (OR: 4.6 [1.4–15] $P = 0.012$) were independent factors significantly associated with NCRD.

None of the factors were significantly associated with LMWH overdosing.

Patients being male (OR: 1.8 [1.03–3.2] $P = 0.041$) and ECOG 3–4 (OR: 2.9 [1.3–6.4] $P = 0.008$) were significantly associated with incorrect injection schedule.

Overall, men (OR: 0.6 [0.4–0.9] $P = 0.019$) and ECOG 3–4 patients (OR: 0.5 [0.2–0.9] $P = 0.025$) were significantly less exposed to NCTR while history of surgery or bleeding were associated with NCTR (OR: 2.2[1.3–3.6] $P = 0.005$ and OR: 5.2[1.4–19] $P = 0.012$, respectively).

Conclusion: Overall, surgery or bleeding history, were independent factors associated with NCTR while poor clinical condition and male gender were associated with a lower risk of NCTR.

1-Farge J Thromb Haemost. 2013 Jan;11(1):56–70.

2-Farge D Blood 2014. 124:1545.

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PO625-MON

Hospital length of stay for deep vein thrombosis or pulmonary embolism among patients receiving rivaroxaban or warfarin

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Background: Venous thromboembolism, including deep vein thrombosis (DVT) and pulmonary embolism (PE) result in substantial health-care system burden. Treatment with warfarin (WAR), unlike the new oral anticoagulants such as rivaroxaban (RIVA), requires monitoring to achieve an international normalized ratio goal, potentially prolonging a patient's hospital length of stay (LOS) and utilization.

Aims: To compare hospital LOS among DVT and PE patients initiating RIVA vs. WAR in a real-world setting.

Methods: Adults with a hospitalization for a primary diagnosis of DVT or PE and initiated on RIVA or WAR were selected for retrospective claims analyses using MarketScan's Hospital Drug Database from 11/01/2012 and 12/31/2013. Patients receiving apixaban, dabigatran, or both RIVA and WAR were excluded. WAR patients were matched 1:1 to RIVA patients using propensity score methods. LOS was analyzed descriptively and modeled using negative binomial regression.

Results: Final matched cohorts each included 751 primary PE and 472 primary DVT admissions. Patient characteristics were well matched on demographic and clinical characteristics. Mean age of the matched cohort was 64.5 years and 51.6% were females. Among the DVT patients, the mean [median] hospital LOS for RIVA patients was 3.7 [3] days comparing to 5.0 [4] days for WAR cohort. The mean difference in DVT hospital LOS of 1.3 days was significant at $P < 0.001$. The mean [median] LOS for primary PE was 3.8 [3] days for RIVA and 5.0 [4] days for WAR. The mean difference in PE hospital LOS of 1.2 days was significant at $P < 0.001$. Modeling confirmed these results. Days from first dose to discharge were 2.41 ± 1.74 (RIVA) and 4.02 ± 3.26 (WAR) for patients initiated with parenteral anticoagulants ($P < 0.001$); and 2.73 ± 1.72 (RIVA) and 3.81 ± 2.31 (WAR) when initiated without parenteral anticoagulants ($P < 0.001$).

Conclusion: For DVT and PE admissions, rivaroxaban was associated with significantly shorter hospital LOS than warfarin treatment.

Disclosure of Interest: S. Deitelzweig Consultant for: Janssen Scientific Affairs, LLC who is the sponsor of this study., M. Raut Employee of: Janssen Scientific Affairs LLC which sponsored the study., J. Margolis Consultant for: Truven Health Analytics, which was paid by Janssen Scientific Affairs in connection with the development of this abstract, O. Tran Consultant for: Truven Health Analytics, which was paid by Janssen Scientific Affairs in connection with the development of this abstract, D. Smith Consultant for: Truven Health Analytics, which was paid by Janssen Scientific Affairs in connection with the development of this abstract, B. Bookhart Employee of: Janssen Scientific Affairs LLC which sponsored the study., J. Schein Employee of: Janssen Scientific Affairs LLC which sponsored the study., W. Olson Employee of: Janssen Scientific Affairs LLC which sponsored the study.

PO626-MON

Acute pulmonary embolism: external validation of the 2014 risk stratification model of the European society of cardiology

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Background: In patients with acute pulmonary embolism (PE), risk stratification for short-term mortality is crucial to drive clinical management. The European Society of Cardiology (ESC) has recently proposed an updated model for risk stratification based on clinical features, right ventricle dysfunction (RVD) and/or elevated troponin (2014-ESC model).

Aims: The aim of this study was to provide an external validation of the 2014-ESC model.

Methods: Consecutive patients with symptomatic, objectively confirmed PE were included in prospective cohorts that were subsequently merged in a collaborative database. Patients were included in the analysis if full information about their sPESI score, RVD (by either echocardiography or computed tomography) and troponin levels were available. Study outcomes were 30-day death and PE-related death (as adjudicated by the local investigator).

Results: Among 906 patients (mean age 68 ± 16 , 489 females), 801 were hemodynamically stable. Death and PE-related death occurred in 7.2% and 4.1% of the patients. Death rates according to risk stratification (2014 and 2008-ESC models) are reported in the Table. One of the 196 low-risk patients died (0.5%). The 2014 and the 2008 ESC models showed similar discriminatory powers for death (c-statistics 0.71; 95% CI 0.65- 0.77 vs. 0.71; 95% CI 0.65-0.78) as well as for PE-related death (c-statistics 0.77; 95% CI 0.70- 0.85 vs. 0.79; 95% CI 0.72-0.85).

Conclusion: The 2014-ESC model avoids further testing in about 20% of the patients preserving a high negative predictive value. The 2014-ESC model has a discriminatory power for death and for death due to PE similar to that of the 2008-ESC model. Further studies are required to improve the clinical profile of patients at intermediate-risk to justify a treatment upgrading.

Disclosure of Interest: None declared.

PO627-MON

Risk factors for recurrence and thromboembolic events after isolated superficial vein thrombosis of lower limbs

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Background: Superficial vein thrombosis (SVT) of the lower limbs is a frequent finding in symptomatic outpatients, but risk factors for recur-

rence and venous thromboembolism (VTE) after stopping treatment are uncertain.

Aims: To assess the three-month outcome of isolated SVT and ascertain the impact of SVT characteristics on outcome.

Methods: In a retrospective single centre study we included all 464 symptomatic outpatients (age 66.5 ± 15.7 year, female 61.9%) in whom a real-time B-mode and color Doppler ultrasonography examination revealed a SVT without deep vein thrombosis (DVT) and/or pulmonary embolism (PE). We excluded SVTs within 3 cm from the sapheno-femoral/popliteal junction, patients with renal insufficiency ($\text{GFR} < 30 \text{ mL min}^{-1}$), and patients that received vitamin K antagonists. Patients received full dose LMWH for 1 week followed by half therapeutic dose LMWH for 3 weeks ($n = 355$) or fondaparinux 2.5 mg for 6 weeks ($n = 109$). The outcome of the present study was symptomatic recurrent SVT, PE, and DVT. Follow-up visits were at 3 months.

Results: The most frequent risk factors for SVT in the study population was venous insufficiency (69.4%), cancer was present in 3.5% of patients. During follow-up, 36 recurrent SVT (rate 7.7%, CI95%: 5.7–10.6%), 10 venous thrombo-embolic events (VTE rate 2.2%, CI95%: 1.2–3.9%) were recorded, which included 2 PE, and 8 DVT. Patients with cancer (OR 8.5, CI95%: 1.6–44; $P = 0.01$) were at higher risk for VTE but not for SVT recurrence. Male patients were at higher risk for SVT recurrence (OR 2.1, CI95%: 1.1–4.3; $P = 0.03$). The type of anticoagulant therapy, age, and the presence of venous insufficiency were not associated with the outcome.

Conclusion: The risk of recurrent VTE after SVT may be relevant, especially in patients with active cancer.

Disclosure of Interest: None declared.

PO628-MON

Efficacy and safety of treatment with anticoagulants in patients with venous thromboembolism in Korea

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Background: The incidence of venous thromboembolism (VTE) in Asia, is 33% lower than that of western populations, but recent retrospective study in Korean population, demonstrated a yearly increasing incidence of VTE. So social concern is rising.

Aims: This study is designed to investigate the efficacy and safety of treatment with anticoagulant in patients with pulmonary embolism (PE) and/or deep vein thrombosis (DVT) in South Korea.

Methods: We retrospectively evaluated the efficacy and safety of treatment of 441 patients, including 139 patients with provoked group and 302 with unprovoked group, consecutively collected from January 2005 to June 2013, in single tertiary hospital in Korea.

Results: Mean age was 66.4 ± 13.3 for provoked group and 68.1 ± 12.2 for unprovoked group. Patients with PE only in provoked group and unprovoked group were 88 (63.3%) and 175 (58%), respectively. Recurrence rate were 10 (7.2%) for provoked group, 41 (13.6%) for unprovoked group, and 51 (11.6%) for overall. P -value for recurrence rate was 0.052. 30-day mortality were 5 (3.6%) for provoked group, 7 (2.3%) for unprovoked group, and 12 (2.7%) for overall.

Conclusion: Recurrence rate was similar to that of western study for provoked and unprovoked VTE patients in Korea respectively. Special care for increasing number of patients with VTE is needed.

Disclosure of Interest: None declared.

Vitamin K antagonists I

PO629-MON

Prevalence of VKORC-1 polymorphisms across caucasians and amerindians

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Background: Vitamin K epoxide reductase complex 1 (VKORC-1) is the target enzyme of vitamin K antagonists. Polymorphisms (SNPs) in VKORC-1 gene, mainly 1173C>T and –1639G>A, are associated with different sensitivity to oral anticoagulants (OA). The knowledge about the frequency of these SNPs in Amerindians is limited.

Aims: The aim of the study was to determine, VKORC-1 SNPs (1173C>T and –1639G>A) frequency across Caucasians and descendants of Amerindians, in order to see possible differences between them.

Methods: Subjects: 130 random patients were selected (40% males and 60% females with mean age of 62 (range: 8–95) year-old; 65 individuals were Caucasian and 65 were descendants of Amerindians. The ethnic origin was assigned according to a questionnaire specially designed and validated for that purpose. Genotyping was performed using RFLPs: PCR reaction, followed by MspI (–1639G>A) or StyI (1173C>T) digestion. Differences in allele frequencies among racial groups were compared with Chi square test.

Results: SNPs frequencies were A) –1639G>A: 20 GG, 27 AG, 18 AA in Caucasians and 16 GG, 36 AG, 13 AA in descendants of Amerindians and B) 1173C>T: 18 CC, 29 CT, 18 TT in Caucasians and 14 CC, 41 CT, 10 TT in descendants of Amerindians. Differences between groups were not significant (Chi square $P = 0.281$ and $P = 0.089$ respectively).

Conclusion: Although the differences between the ethnic groups analyzed were not significant, higher proportion of heterozygotes were found in descendants of Amerindians for both SNPs. The number of subjects included in this preliminary analysis could be insufficient to show significant differences. Moreover, the method applied to assign the ethnic characteristics would imply some degree of ethnic misclassification. In order to exclude possible differences between these groups we think that the analysis should be done on a higher number of subjects in whom ethnic assignment is performed by short tandem repeat (STR) markers.

Disclosure of Interest: None declared.

PO630-MON

Influence vitamin K antagonists on the thrombinemia and homocysteine levels

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Background: Vitamin K antagonists administered for the prevention and treatment of thrombosis.

Aims: The aim to determine the thrombin, thrombin – antithrombin complex, homocysteine levels at patients with atrial fibrillation and deep vein thrombosis, taking warfarin.

Methods: The study included 46 patients, men and women at the age of 54 (21 to 75 years) year, taking vitamin K antagonists. First group consisted the patients with deep venous thrombosis ($n = 37$), the second group – patients with atrial fibrillation ($n = 9$). All content was determined in serum indicators thrombinemia level: thrombin, «thrombin – antithrombin» complex, and a marker of endothelial dysfunction – homocysteine 1-st day (baseline) and at the 6-th month of warfarin therapy.

Results: The initial values of the studied parameters were not significantly different between groups, except for the «thrombin-antithrombin» complex level, which was higher in patients with deep venous thrombosis ($P < 0.05$). After six months of use of warfarin the thrombin level were decreased statistically significantly in patients with atrial fibrillation ($P < 0.05$) and «thrombin – antithrombin» complex level did not change ($P > 0.05$). In patients with deep vein thrombosis thrombin level did not decrease ($P > 0.05$), and «thrombin-antithrombin» complex level increased statistically significantly ($P < 0.05$). Homocysteine levels in both groups remained a high, due to the forced use of a diet devoid green plants and vegetables, i.e. deficient in folic acid and B vitamins.

Conclusion: Thus, patients of middle age with atrial fibrillation and with thrombosis vitamin K antagonists therapy do not the level of homocysteine knobjockey diet to maintain the effectiveness of therapy. Patients with atrial fibrillation therapy vitamin K antagonists therapy leads to lower of thrombin levels. The content «thrombin-antithrombin» complex higher in patients with thrombosis, due to original higher thrombin level.

Disclosure of Interest: None declared.

PO631-MON

Susceptibility of VKORC1 and its paralog VKORC1L1 towards different 4-hydroxycoumarins

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Background: Vitamin K 2,3-epoxide reductase complex subunit 1 (VKORC1) and its paralog VKORC1-like1 (VKORC1L1) are enzymes which catalyze the reduction of vitamin K (VK) 2,3-epoxide to VK quinone and further to VK hydroquinone. However, they differ in their substrate affinities and physiological roles. VKORC1 serves VK hydroquinone for gamma carboxylation of vitamin K dependent proteins, whereas VKORC1L1 was found to play a central role in intracellular antioxidation. Both enzymes can be inhibited by Warfarin and other 4-hydroxycoumarins (4-HC).

Aims: In the present study half-maximum inhibitory concentrations (IC50 values) of different 4-hydroxycoumarins were determined for the enzymes VKORC1 and VKORC1L1, respectively.

Methods: VKORC1 and VKORC1L1 gene expression was knocked out in HEK cells using the CRISPR/Cas9 gene editing technique. Positive clones were verified through next generation sequencing (NGS) and mRNA analysis. The cell-based assay was performed as described by Fregin et al (2013). Different concentrations (ranging from 0.05 to 500 nM) of therapeutically used coumarins and various rodenticides were tested and FIX activity was plotted against the respective 4-HC concentration.

Results: MiSeq NGS revealed a 19 bp deletion (concomitant with a substitution) in the VKORC1 gene and a two bp deletion and single bp insertion in the VKORC1L1 gene. The altered genomic sequence was confirmed by mRNA analysis. In the cell-based assay Warfarin showed the highest IC50 difference between endogenous VKORC1 and VKORC1L1, 3.2 nM and 135 nM, respectively. The rodenticides however, showed no marked difference in IC50 values.

Conclusion: VKORC1 and VKORC1L1 are enzymes susceptible to 4-HC, with the therapeutically used coumarins showing a higher affinity on VKORC1. Additionally, low levels of Warfarin inhibit VKORC1 whereas inhibition of VKORC1L1 requires a more than 40-fold increase in Warfarin concentration. In contrast, rodenticides like bromadiolone are unselective inhibitors of the complete VK metabolism.

Disclosure of Interest: None declared.

PO632-MON

Zymogen-like FXa variant as novel warfarin reversal strategy: pre-clinical evaluation and mechanism of action

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Background: Despite emergence of novel oral anticoagulants, warfarin remains the mainstay of thromboembolism intervention. It prevents γ -carboxylation of vitamin K-dependent clotting factors, altering protein-membrane binding required for coagulation. We previously developed a zymogen-like FXa molecule, FXa^{116L}, with impaired active site maturation and a greater half-life than wild-type FXa, but with normal procoagulant function in the prothrombinase complex.

Aims: We hypothesized FXa^{116L} would achieve *in vivo* hemostasis after injury in mice anticoagulated with warfarin via bypassing affected factors upstream of prothrombin and directly activating prothrombin to thrombin.

Methods: A carotid artery injury model (7.5% FeCl₃) was employed to evaluate hemostasis in the presence or absence of FXa^{116L}. Complete vessel occlusion after injury was considered hemostatic. Untreated and warfarin anticoagulated mice served as negative and positive controls, respectively. Experimental warfarin anticoagulated mice of INR cohorts 2–3, 6–8 or >8 were administered FXa^{116L} 5 min after injury.

Results: In untreated mice, occlusion occurred 6–8 min after injury while warfarin anticoagulated mice did not occlude after 30 min. In contrast, vessel occlusion in all warfarin mice occurred 3–7 min after FXa^{116L} administration, irrespective of INR.

Conclusion: Our results provide the first *in vivo* efficacy data for use of FXa^{116L} to reverse warfarin-associated coagulopathy and support its use as a quick onset, short acting warfarin reversal agent. *In vivo* data corroborate *ex vivo* results in which FXa^{116L} restored warfarin anticoagulated human whole blood thromboelastogram parameters. The ability of FXa^{116L} to restore *in vivo* hemostasis in a warfarin mouse model suggests it can activate un/undercarboxylated prothrombin and generate sufficient thrombin to achieve hemostasis. This contrasts convention and questions the absolute requirement of prothrombin γ -carboxylation for robust thrombin generation. FXa^{116L} is in clinical development.

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PO633-MON

Clinical audit of anticoagulation reversal and outcome in warfarin-associated intracranial haemorrhage (WAICH)

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Background: WAICH has been documented at a rate of 1.48% in the first 3 months and 0.65% year⁻¹ thereafter. WAICH is associated with a 30-day mortality rate of 59.2%, however there is emerging data that this may be reduced by the prompt reversal of the anticoagulant effect of warfarin. There is little published Australian data regarding the effectiveness of current practice in reversing warfarin in acute WAICH.

Aims: To assess the timeliness and completeness of warfarin reversal in patients with WAICH, the consistency of current practice with consensus guidelines, and the impact of these factors on patient outcome.

Methods: We conducted a dual centre retrospective analysis of patients presenting between July 2009 and June 2014 with WAICH. Patients

were identified using the South Australian Blood Utilization Database. Clinical, laboratory and imaging data, including timeliness of reversal and in-hospital mortality were obtained by review of electronic and paper patient records.

Results: In total, 282 patients were identified (57% male). The preliminary analysis was done on 175 patients (mean age 79 years). Intra-parenchymal haemorrhage accounted for 35% of cases. Concurrent use of an antiplatelet agent was present in 7.4% of patients. Median INR on presentation was 2.6. Mean time from triage to CT scanning was 5.1 h and CT to administration of prothrombin complex concentrate, FFP and vitamin K were 4.2 h, 5.0 h and 6.5 h respectively. 6.2%, 10.5% and 21% of patients had an INR ≤ 1.3 at 2 h, 4 h and 6 h post time of presentation and 81.5% at 24 h. When INR was corrected within 2 h (6.2% of patients), 30-day mortality rate was 0%. Amongst all cases, 30-day mortality was 19%. Twenty-four of 80 cases reviewed by paper records (30%) received therapy consistent with the guidelines. Complete analysis for all the patients will be available for final presentation.

Conclusion: Delays in delivery and divergence in practice from the published guidelines may decrease or negate the potential benefits of the reversal process.

Disclosure of Interest: None declared.

PO634-MON

Effect of INR precision and accuracy on therapeutic quality control in patients receiving vitamin K-antagonists

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Background: Therapeutic Quality Control (TQC) of anticoagulation consists of continuous assessment of the proportion of time spent by each patient in the target range. TQC of an anticoagulation clinic may be approximated by the proportion of INRs in the therapeutic range. To obtain this proportion, it is recommended to review all patients' last INR values at a certain point in time, i.e. one INR value per patient (Thromb Haemost 1990;63:316). TQC depends, in part, on the quality of laboratory or point-of-care (POC) INR measurements. In general, laboratory measurements are more precise than POC measurements. Also, values for the International Sensitivity Index (ISI) of a system may vary by approximately 5% from the true values.

Aims: To assess the effect of INR precision and accuracy on TQC.

Methods: A theoretical model was used in which the INRs of patients were normally distributed. The percentage of INRs within the therapeutic range (2.0 – 3.0) was used as a measure of TQC. TQC was calculated for various values of measurement imprecision and inaccuracy. Imprecision was expressed as the coefficient of variation (CV, in percent). A systematic error in the ISI of a measurement system was used to calculate the effect of INR inaccuracy on TQC.

Results: TQC decreased with increasing imprecision. For example, if the true TQC was 60.5% (i.e. when the INR mean and standard deviation of the distribution was 2.5 and 0.595, respectively) the apparent TQC for CV = 4% was decreased to approximately 60.0%. For CV = 8%, TQC was further decreased to 58.2%. For a systematic error of 5% increase of the ISI, TQC was decreased to 55%. For a systematic error of 5% decrease of the ISI, TQC was increased to 63.6%.

Conclusion: For imprecision equal to or less than 4% there was no important effect on TQC. The effect of systematic ISI errors on TQC depends on the sign and the magnitude of the ISI error.

Disclosure of Interest: None declared.

PO635-MON

Hemizygous deletion of 7Q11.21-Q11.23 eliminating VKORC1L1 does not impair blood coagulation but reveals accumulation of menaquinone-4

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Background: The warfarin-sensitive vitamin K epoxide reductase encoded by VKORC1 regenerates vitamin K hydroquinone to ensure γ -carboxylation of the vitamin K-dependent coagulation proteins. A paralogous protein encoded by VKORC1L1 shares 50% sequence identity with VKORC1 and reduces vitamin K epoxide *in vitro*. The biological role for VKORC1L1 remains unknown.

Aims: A patient with infantile spasms underwent array-Comparative Genomic Hybridization demonstrating a 13 Mb deletion of 7q11.21-q11.23 resulting in hemizygous deletion of VKORC1L1. We aimed to clarify the role of VKORC1L1 by studying its partial deficiency in humans for the first time.

Methods: Citrated plasma was used to determine the prothrombin and partial thromboplastin times, specific blood coagulation protein activity levels, and des- γ -carboxy prothrombin level. Vitamin K analysis was performed by reversed-phase HPLC with post-column reduction followed by fluorometric detection.

Results: Coagulation parameters were normal with a prothrombin time of 11.0 s, international normalized ratio 1.0, and partial thromboplastin time 35.5 s. Vitamin K-dependent protein levels were unaffected with prothrombin 114%, factor VII 84%, factor IX 81%, and factor X 75%. Plasma des- γ -carboxy prothrombin was < 2.0 ng mL⁻¹. Plasma demonstrated significant accumulation of menaquinone-4, a metabolite of vitamin K1, to 0.5 nM. Menaquinone-4 was not detected in pooled normal plasma.

Conclusion: Hemizygous deletion of VKORC1L1 does not impair γ -carboxylation of vitamin K-dependent clotting proteins or coagulation in humans. Deletion of 7q11.21-q11.23 reveals unanticipated accumulation of menaquinone-4 but not accumulation of the quinone epoxide forms. These findings suggest that VKORC1 and VKORC1L1 are not redundant, but may prefer phyloquinones and menaquinones, respectively. Our findings help to explain the exclusive identification of mutations in VKORC1 predicting warfarin dose requirement and causing vitamin K-dependent clotting factor deficiency type II.

Disclosure of Interest: None declared.

PO636-MON

Selection, management and outcome of VKA patients with atrial fibrillation not switched to novel oral anticoagulants – results from the Dresden Noac registry (NCT01588119)

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Background: Currently, many atrial fibrillation (AF) patients are switched from vitamin-K antagonists (VKA) to non-VKA oral anticoagulants (NOAC).

Aims: To evaluate patient characteristics, selection patterns and outcome of VKA patients with AF not switched to NOAC in daily care.

Methods: Then the Dresden registry stopped enrolment of NOAC patients with AF on February 28, 2013 participating physicians were asked to enrol all VKA patients seen for regular INR testing, who remained on VKA at that day and for whom switching to NOAC was not anticipated. VKA patients were followed until October 31th 2014.

Results: The sites that enrolled 427 VKA patients had previously enrolled 706 NOAC patients, which served as control. Demographic characteristics were comparable for both cohorts but VKA patients had a significantly lower bleeding risk (HAS-BLED score ≥ 2 : 38.2% VKA vs. 60.6% NOAC patients).

Mean duration of VKA pre-treatment was 72 months (range 3–384 m). Stable INR was the predominant reason (95.3%) for VKA continuation. Before enrolment, mean ($\pm$ SD) time in therapeutic INR range (TTR) was 71% ($\pm$ 21).

During a median follow-up of 475d, TTR increased to 75%. 5 VKA patients (1.2%) experienced stroke/TIA/systemic embolism during or within 7 days after last VKA intake (0.94/100 patient-years; 95% CI 0.30– to 2.19). 22 patients (5.2%) patients experienced ISTH major bleeding (4.15/100 patient-years; 95% CI 2.60– to 6.29).

In contrast, 18 of the 706 NOAC patients (2.6%) treated at the same sites (median follow-up of 730 d) developed stroke/TIA/systemic embolism during or within 3 days after their last intake of NOAC treatment (1.5/100 patient-years, 95% CI 0.9–2.39) and 44 patients (6.2%) patients experienced ISTH major bleeding (3.74/100 patient-years; 95% CI 2.72–5.02).

Conclusion: Patients with stable INR and low bleeding risk are selected for VKA continuation. With a TTR of 75%, cardioembolic events are rare but the rate of major bleeding is at least as high as that of patients at higher bleeding risk treated with NOAC.

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PO637-MON

Antagonization of warfarin in cells: warfarin binding on human VKORC1 is reversible

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Background: Vitamin K Oxidoreductase Complex subunit 1 (VKORC1) catalyzes the reduction of vitamin K 2,3-epoxide to the quinone form and further to vitamin K hydroquinone (KH₂). KH₂ serves as essential substrate during γ -carboxylation of vitamin K dependent proteins (e.g. FII, FVII, FIX, FX) by γ -glutamyl carboxylase. In therapy and prevention of thrombosis, inhibition of VKORC1 by 4-hydroxycoumarins like Warfarin are used for oral anticoagulation. Warfarin is thought to bind VKORC1 irreversibly. However, over-anticoagulation can be antagonized *in vivo* by high doses of vitamin K. The mechanism for this effect is still unsolved

Aims: Characterization of Warfarin binding to VKORC1 in a cell-based system.

Methods: HEK293T knockout (KO) cell lines for *VKORC1* and *VKORC1L1* were generated by CRISPR/Cas9 gene editing. These cells were transfected with *F9* cDNA, or a bicistronic vector containing *VKORC1* and *F9* cDNA. Before and after transfection cells were grown in media with different concentrations of Warfarin and vitamin K₁ (VK₁). FIX activity served as surrogate marker for VKORC1 activity and Warfarin inhibition

Results: All cell lines showed decreased FIX activities when treated with Warfarin. FIX activity was recovered at all Warfarin concentrations by increasing VK₁ levels. E. g. *VKORC1L1* KO cells treated with 5 nM Warfarin and 5 ng μ L⁻¹ VK₁ showed residual FIX activity of 30%. Supplementation of a higher VK₁ concentration of 25 ng μ L⁻¹ at 5 nM Warfarin led to an increase in FIX activity up to 60%. *VKORC1/VKORC1L1* double KO cells transfected with *F9* cDNA did not show any FIX activity when treated with high VK₁ concentrations

Conclusion: Warfarin binding to VKORC1 is reversible with VK₁ able to displace Warfarin. The high VK₁ levels needed to recover FIX activity reflects the higher affinity of Warfarin to VKORC1 compared to VK₁. Additionally, there is no bypass enzyme present catalyzing vitamin K reduction in double KO cells. Thus, antagonization of overdosed Warfarin in patients by vitamin K probably follows the same mechanism

Disclosure of Interest: None declared.

PO638-MON

Relationship between vitamin k antagonists and recurrent bleeding events in patients with non-valvular atrial fibrillation: real-world findings from England

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Background: More than one million adults in the UK are affected by non-valvular atrial fibrillation (NVAf), a leading cause of arterial embolisation and stroke. The most commonly prescribed anticoagulant in the UK is a vitamin K antagonist (VKA).

Aims: Estimate the association between bleeding events on the risk of subsequent bleeding in patients with NVAf anticoagulated with VKA.

Methods: Retrospective cohort study of NVAf patients treated with VKA using Clinical Practice Research Datalink and Hospital Episode Statistics databases (CPRD-HES), containing GP and hospitalization data in England. Using Read, ICD-10, and Product codes to identify NVAf, bleeds (any bleeds [AB] as a composite of major bleeds [MB] and clinically relevant non-major bleeds [CRNMB]), and VKA prescriptions. We used time-varying Cox models to estimate the risk of another bleed after each bleeding event, reported as hazard ratios (HRs) and 95% CIs adjusted on baseline patient characteristics, during follow-up time on VKA therapy.

Results: We identified 29,489 NVAf patients treated with VKA. Median follow-up time was 22 patient-months. Mean age was 73.4 (SD 10.5), 58% were men, and 82% were Caucasian. Overall, 14.6%, 8.1%, and 6.5% of patients experienced at least one AB, MB, or CRNMB, respectively. Among patients who continued on VKA after a 1st bleed, 35.3%, 16.2%, and 19.2% suffered at least a 2nd AB, MB, or CRNMB, respectively. We observed a 4-fold increased risk of a recurrent bleed following a 1st bleed (HR 4.2; 3.9–4.5) and a 5-fold to 8-fold increased risk of 2nd (HR 5.6; 5.0–6.2) and 3rd (HR 8.4; 7.4–9.6) bleeds compared to follow-up time without any bleed.

Conclusion: One in 7 NVAf patients treated with a VKA suffered a MB or CRNMB, which was predictive of their risk of suffering subsequent bleeds on VKA. There is a clear need for further research to

guide patient bleed risk stratification as well as the role of VKA discontinuation and/or initiation of alternative anticoagulation therapies.

Disclosure of Interest: R. Alikhan Consultant for: Bristol-Myers Squibb, Pfizer, Bayer, Boehringer Ingelheim, and Daiichi Sankyo, C. Lefevre Employee of: Bristol-Myers Squibb, I. Menown Consultant for: Bristol-Myers Squibb, Pfizer, Bayer, and Boehringer Ingelheim, S. Lister Employee of: Bristol-Myers Squibb, L. Lacoine Employee of: Bristol-Myers Squibb, A. Bird Employee of: Pfizer, M. You Employee of: Bristol-Myers Squibb, E. Ridha Employee of: Bristol-Myers Squibb, D. Evans Employee of: Bristol-Myers Squibb.

PO639-MON

Risk factors for gastro-intestinal bleeding in atrial fibrillation patients on VKA treatment: prospective analysis of two cohorts of patients

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Background: Vitamin K antagonists (VKA) therapy for stroke prevention in atrial fibrillation (AF) has been the treatment of choice until the marketing of novel anticoagulants (NOACs). A reduction of cerebral bleeding has been demonstrated with NOACs, instead VKA showed a lower gastro-intestinal(GI) bleeding risk.

Aims: We examined 2 cohorts of AF patients to evaluate risk factors associated with GI bleeding. **Methods:** The study included 3015 AF patients enrolled in EPICA study(1)(cohort 1) and 1085 AF patients previously described(2) (cohort 2). Risk factors for GI bleeding were analyzed at univariate analysis

Results: Cohort 1 patients were older (median age 83(80–102)years) than cohort 2 (median age 75(30–94 years); 51 ad 33 GI bleedings (rate 0.67 and 0.66x100 patients/years) were recorded in cohort 1 and 2, respectively. Only history of bleeding (table) was significantly associated to bleeding risk at Cox regression analysis in both cohorts

	Cohort1 GI	Cohort1 No GI	p value	Cohort2 GI	Cohort2 No GI	P value
Sex(female)	30	1331	0.06	15	395	0.36
Hypertension	41	2158	0.24	23	734	1.00
Diabetes	11	515	0.45	5	219	0.51
CAD	19	654	0.01	4	234	0.20
Renal failure	4	296	1.00	2	71	1.00
Previous stroke/tia	14	577	0.15	13	299	0.17
Previous bleeding	11	75	0.000(*)	4	29	0.03(**)
MedianTTR (IQR)	65 (49–77.7)	63 (50–75)	0.78	72 (64.5–76)	74 (62–82)	0.54
Heart failure	17	768	0.25	9	259	0.65

(*)OR 13.5 (95% CI 6.8–27.0) (**) OR 3.1 (95%CI 1.1–8.7)

Conclusion: In this study on AF patients on VKA the risk of GI bleedings was similarly low among very old and younger patients. The history of previous bleeding events was the only risk factor associated with GI bleeding.

(1) Poli D, et al. Circulation. 2011;124:824–9

(2) Poli D, et al. Intern Emerg Med. 2014;9:443–7

Disclosure of Interest: None declared.

PO640-MON

Predictors of ich recurrence on vka: severity of the first episode and hasbled score fail to identify high risk patients from the CHIRONE Study

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Background: The most feared complication of Vitamin K Antagonists (VKAs) treatment is intracranial hemorrhage (ICH). The previously published CHIRONE Study (1) fails to identify risk factors associated with ICH recurrence after VKAs resumption

Aims: The aim of this secondary analysis of the Study is to evaluate if patients who need surgery or with severe neurological sequelae after the first episode show a higher risk of ICH recurrence. The HASBLED score was used to

Methods: The study included 267 patients from 27 Italian Centres. The treatment of the index ICH, surgical or medical was recorded; Modified Rankin Scale (mRS) score ≥ 3 was used to define patients with severe neurological impairment; HASBLED score ≥ 3 was used to identify high bleeding risk patients

Results: During follow-up, 20 patients (7.5%) had ICH recurrence (rate of 2.56 x100 pt-yrs). No difference in the type of treatment (HR 1.5; 95% CI 0.49–4.74) and neurological impairment with mRS ≥ 3 (HR 0.9; 95% CI 0.31–2.83) were found in relation to ICH recurrence. The mean HASBLED score was similar between the 2 groups ($P = 0.54$).

Conclusion: In conclusion the results of our study suggest that neither the severity of the index ICH at presentation nor the HASBLED clinical prediction rule should be used to assess the risk of recurrence in patients who need VKAs resumption after a previous ICH.

(1)Poli D, Antonucci E, Dentali F, Erba N, Testa S, Tiraferri E, et al. Recurrence of ICH after resumption of anticoagulation with VK antagonists: CHIRONE study Neurology. 2014 25;82:1020–1026

Disclosure of Interest: None declared.

PO641-MON

Perioperative interruption of warfarin anticoagulation

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Background: In the absence of data from randomized controlled studies, the 2012 ACCP guidelines do not recommend using low molecular weight heparin (LMWH) bridging anticoagulation therapy in patients with low risk atrial fibrillation (AF), defined as CHADS 2 score of 0–2 (including no stroke in the past 6 months), who require interruption of warfarin for surgery or procedures.

Aims: To audit clinical management of perioperative warfarin interruption in patients with low risk AF in a practice of ten thrombosis physicians.

Methods: A prospective audit evaluating two four-month periods between 2013 and 2014 was performed at The Ottawa Hospital. The main outcome was use of bridging for perioperative management of anticoagulation according to ACCP guidelines. Other outcomes assessed were arterial and venous thrombotic events and major bleeding events up to 30 days post-procedure.

Results: In this time period 235 patients on warfarin anticoagulation were evaluated for perioperative anticoagulation management. Of this, 56 patients met criteria for low risk AF. The prescribed bridging regimen follows: 19 patients (34%) received LMWH pre- and post-operatively; 16 (29%) had pre-op LMWH only; 3 (5%) had post-op LMWH only; and 18 patients (32%) received no LMWH bridging during warfarin interruption. All postoperative doses of LMWH prescribed were prophylactic doses. One patient receiving LMWH bridging suffered a stroke on post-operative day one. There were no adverse bleeding or venous thrombotic events.

Conclusion: In this single centre evaluation of perioperative anticoagulation management, less than one third of patients received treatment in accordance with current clinical practice guidelines, which suggests avoidance of bridging. Well-designed studies evaluating the efficacy of LMWH are needed to reduce variation in practice and avoid the exposure of patients to potentially harmful interventions.

Disclosure of Interest: None declared.

Von Willebrand disease I

PO642-MON

Cost of treating thrombotic events in a US population of von Willebrand disease patients

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Background: Current available factor replacement therapy (FRT) for von Willebrand Disease (vWD) contains FVIII. Thromboembolic events (TEE) are a small but serious risk in patients with elevated FVIII levels as a result of accumulation from repeated doses of FRT (Manucci, 2004).

Aims: To assess the total medical cost incurred in one year of treating TEEs in vWD.

Methods: This retrospective database analysis utilized the MarketScan® Database, a US medical claims database, from Jan 2006 to Mar 2014. Patients with inpatient diagnosis for a TEE (ICD-9 code for venous thromboembolism [VTE], myocardial infarction [MI], ischemic stroke [IS], etc.) were identified, with the first inpatient TEE visit defined as the index date (ID). VWD diagnosis (286.4) was required prior to ID and ≥12 month continuous enrollment both prior and post ID. Direct medical costs of TEE management in the first year were estimated for inpatient and outpatient settings. The subset of VWD

patients prescribed FRT prior to ID was also described. Costs were adjusted to 2013USD using the medical consumer price index.

Results: One hundred fifty-three vWD subjects were identified with an inpatient TEE. Mean age at the ID was 56 years (2–94 yrs) and 69.9% were female. Median annual cost and range associated with treatment of TEEs was \$21,166 (\$1300–\$867,710). VTE and IS were most common TEEs (36.0% ≥1 VTE, \$24,338 (\$1063–\$235,090) and 32.7% ≥1 IS, \$11,326 (\$1300–\$368,550), respectively). Patients prescribed FRT ($n = 12$) had a median annual cost of \$64,648 (\$4690–\$137,705) for TEE treatment.

Conclusion: This is the first study to estimate the total cost associated with management of TEEs in vWD. TEEs are a burden to the health-care system and are potentially life-threatening. A vWD treatment option that can avoid the risk of FVIII accumulation may benefit vWD patients at risk for TEEs.

Disclosure of Interest: A. Khachatryan Employee of: Baxter Healthcare, Y. Xiong Employee of: Baxter Healthcare.

PO643-MON

An open-label, multi-center extension study to assess the efficacy and safety of a plasma-derived von willebrand factor/factor VIII (VWF/FVIII) concentrate in pediatric, adolescent, and adult subjects with von willebrand disease

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Background: Voncento® comes with a low-volume and the solution contains a large proportion of high-molecular-weight VWF multimers with a VWF:FVIII ratio of 2.4:1. The SWIFT ('Studies with von Willebrand factor/Factor VIII') program is evaluating this product in hemophilia A and VWD patients in accordance with the European clinical and pediatric guidelines.

Aims: This open-label study evaluated the long-term efficacy and safety of Voncento® in VWD subjects in whom treatment is required for prophylactic therapy, hemostatic control during surgery, or control of a non-surgical, spontaneous, or traumatic bleeding event.

Methods: Twenty subjects (3 children aged <12 years from the pediatric SWIFTLY-VWD study; 15 adults (≥18 years) and 2 adolescents (12–<18 years) from SWIFT-VWD study) who had completed their 12 month treatment period extended their treatment up to an additional 32 months.

Results: In the prophylaxis arm, 9 subjects received a median number of 109 infusions (median dose 43 IU VWF:RCO kg⁻¹). They reported 118 non-surgical bleeds (NSB), 96 (71%) of which required treatment; 1 subject had no bleeds. The majority of NSB required only 1 infusion (71%) and were minor. Hemostatic efficacy was assessed by the investigator as excellent or good for 98% of bleeds. In the on-demand arm 8 subjects experienced 402 NSB (median number 35), 77 of which required treatment. The median number of infusions was 17 (median dose 55 IU VWF:RCO kg⁻¹). Hemostatic efficacy was assessed by the investigator as excellent or good for 99% of bleeds. Two subjects started with prophylaxis treatment, but were switched to on-demand therapy. The adverse events were only mild-moderate and

no cases of anaphylactic reactions or angioedema, FVIII/VWF inhibitors, thromboembolic events, or viral infectious disorders were seen.

Conclusion: This contemporary comprehensive development program evaluating Voncento[®] across all ages demonstrates long-term safety and efficacy for treatment and prevention of bleeds in subjects with severe VWD.

Disclosure of Interest: T. Lissitchkov: None Declared, A. Klukowska: None Declared, K. Kuliczowski: None Declared, E. Buevich: None Declared, G. Auerswald: None Declared, O. Stasyshyn: None Declared, W. Seifert Employee of: CSL Behring.

PO644-MON

Thrombocytopenia during DDAVP test in two children with type 2A von Willebrand disease

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Background: Transient thrombocytopenia following DDAVP is not frequent but is reported in patients with von Willebrand disease (VWD) type 2B. Also, serendipitous findings reported this behavior in other patients without diagnosis of VWD 2B. Mutations in von Willebrand factor (VWF) affecting disulfide bridges in the A1 loop are described as type 2A VWD.

Aims: To describe two patients who showed transient thrombocytopenia during DDAVP test who initially presented with type 2A VWD.

Methods: Two unrelated girls (6.3y and 9.0y) with a phenotypic diagnosis of type 2A VWD, both presenting with low VWF:Ag (22 and 27%), VWF:RCO <5% and VWF:CB <5%. No history of thrombocytopenia or hyper responsiveness to low dose ristocetin (LDR). DDAVP testing was performed. Platelet count (PC) and morphology along with PT, aPTT, FVIII:C, VWF:Ag, VWF:RCO, VWF:CB were evaluated at 0, 30, 60, 120 and 240 min post DDAVP infusion. Analysis of VWF exon 28 was performed with PCR-sequencing.

Results: PC decreased from 194 to 27x10⁹L⁻¹ and 274 to 52x10⁹L⁻¹ in both patients at 30 min, with normalization at 120 min. At 30 min, platelet clumps were observed. Slight increases in VWF:CB were evident, although no VWF:RCO amelioration could be demonstrated. Spontaneous aggregation and enhanced response to LDR were detected in mixing studies at this time. Sequencing detected a p.Cys1272Trp mutation in one patient and a p.Cys1458Tyr in the other, affecting the disulfide bridge of the A1 loop of VWF.

Conclusion: We describe two patients with phenotypic data initially compatible with a type 2A VWD who showed a type 2B VWD phenotype during DDAVP testing. To our knowledge, this is the first report of *in vivo* behavior of these mutations. These cases allow us to hypothesize that disruption of A1 disulfide knot lead to a misfolding of the VWF molecule affecting its function and could explain the transient thrombocytopenia in these patients due a rapid binding of VWF to platelet GPIb and a short half-life because of enhanced A2 site exposure to protease action.

Disclosure of Interest: None declared.

PO645-MON

Ruling out von Willebrand disease in preoperative patients with bleeding symptoms

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Background: von Willebrand disease (vWD) is the most common congenital bleeding disorder. Because of the risk of major hemorrhage, timely detection of patients with vWD prior to surgery is imperative. The Platelet Function Analyzer-100 (PFA-100) has been proposed as a screening tool for vWD.

Aims: To determine the sensitivity of the PFA-100 to detect low von Willebrand factor antigen (vWF:Ag) and activity (vWF:RCo) in pre-operative patients with bleeding symptoms.

Methods: Consecutive patients visiting the anesthesiology department from August 2013 to November 2014 reporting ≥ 1 bleeding symptom (s) were included. Exclusion criteria were: referral to the hematology department, antithrombotic drugs, known bleeding disorder, thrombocytopenia or anemia. Citrated blood was obtained before surgery. PFA-100 closure times (CTs) were measured with collagen-adenosine diphosphate (C-ADP) and collagen-epinephrine (C-epi) cartridges, 1 h after blood withdrawal. vWF:Ag, vWF:RCo (Siemens, Marburg), factor VIII and platelet count were measured once. C-ADP > 118 and C-epi > 160 s were considered prolonged. Low vWF:Ag and vWF:RCo were defined as < 50% (reference range 50–150%), warranting further diagnostic steps to diagnose or exclude vWD. Informed consent was obtained and the local medical ethics committee approved the study.

Results: Blood samples were obtained in 146 patients. In 3 patients both vWF:Ag and vWF:RCo were < 50% and in 1 patient vWF:RCo was < 50% and vWF:Ag 52%. Two of these patients had prolonged CT(s). In 13 patients with normal vWF:Ag and vWF:RCo one or both CTs were prolonged. No patients had factor VIII < 60% or platelet count < 150x10⁹ L⁻¹. The sensitivity, specificity, positive and negative predictive value of the PFA-100 for low vWF:Ag and/or vWF:RCo were 50%, 90.8%, 13.3% and 98.5%, respectively.

Conclusion: In preoperative patients with low suspicion of vWD, the PFA-100 is not a reliable test to rule out (mild) vWD as the sensitivity for detecting low vWF:Ag and vWF:RCo is low.

Disclosure of Interest: None declared.

PO646-MON

Health-related quality of life among von willebrand disease patients is not related to disease severity

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Background: von Willebrand disease (VWD) is the most common inherited bleeding disorder known in humans: while estimated to affect between 0.1 and 1% of the general population, many are never diagnosed. Currently, there is paucity of published studies investigating the health-related quality of life (HRQoL) in VWD, particularly among mild VWD patients.

Aims: To determine the HRQoL and its predictors among patients with mild, moderate and severe forms of VWD.

Methods: Patients with clinical diagnosis of VWD were recruited from a tertiary Inherited Bleeding Disorder Clinic in Kingston, Ontario. Upon informed consent, standardized bleeding scores using the Self-

BAT (Self-administered Bleeding Assessment Tool) were obtained. Each participant then completed a HRQoL questionnaire (SF-36). Analyses included paired samples t-tests and Pearson correlations.

Results: 92 patients completed the questionnaires (consent rate=98%). On average, the participants were 38 years of age (range: 18–73), primarily female (80%), and the majority were diagnosed with VWD Type 1 (82%). Compared to age- and sex-matched Canadian normative data, VWD patients had clinically and statistically significant reductions in 7 of 8 HRQoL domains, including physical and mental component summaries. No correlation was found between HRQoL status and VWD severity, bleeding scores, serum ferritin, serum VWF, socioeconomic status or rurality.

Conclusion: This study is the first in North America to examine the impact of VWD on HRQoL across disease severity and bleeding phenotype. Significant reductions in HRQoL scores among VWD patients strengthen the rationale for screening strategies for detection of this under-diagnosed disease in the community. Patients with mild VWD had similar impairment in HRQoL compared to their counterparts with moderate or severe forms of the disease.

Disclosure of Interest: None declared.

PO647-MON

Plasma-derived, purified, pasteurised von Willebrand factor/factor VIII concentrate in the treatment of patients with von Willebrand disease and haemophilia a: update of a long-term observational study

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Background: Comprehensive, large-scale, non-interventional studies are effective tools to collect data on products in the post marketing authorisation period.

Aims: This study assessed the long-term effectiveness, tolerability and safety of a purified, pasteurised and lyophilised human plasma-derived von Willebrand factor/factor VIII concentrate (Haemate[®] P, CSL Behring GmbH, Marburg, Germany) in the treatment of patients with von Willebrand disease (VWD) and haemophilia A (HA).

Methods: Previously untreated and previously treated patients with VWD or HA at any age, who had received Haemate[®] P were eligible for enrollment. Based on the proceeding at the centres, patients were routinely seen and documented every 6 to 12 months. Parameters documented comprised effectiveness-, safety-, pharmacoeconomic-, pharmacokinetic data, relevant concomitant diseases and medication.

Results: Up to now, 108 patients were enrolled into this study and data from 497 visits were available for this analysis. 101 patients suffered from VWD (66 patients with type 1, 23 patients with type 2, 11 patients with type 3, and one patient with unknown type), and 7 patients from HA. The median age was 33 years (range 0.3 – 84.3). In all VWD patients the median number of bleeds per year was 1.80 (in the VWD type 1 patients: median 2.73 bleeds; VWD type 2 patients: median 1.67 bleeds; VWD type 3 patients: median 0.97 bleeds). This is correlated to a higher percentage of patients with on-demand treatment in type 1 patients. Effectiveness of Haemate[®] P was judged to be excellent or good in 93.7% of all bleeds. Of 7 patients with HA, one patient with known risk factors developed a low titer inhibitor that was successfully eliminated by an ITI. There were no thromboembolic

events and no proven virus transmission for hepatitis A/B/C or human immunodeficiency virus.

Conclusion: The results included in this interim analysis confirm the very good efficacy, tolerability and safety of Haemate[®] P.

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PO648-MON

Evaluation of an elisa based assay for detection of type 2N von Willebrand disease

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Background: Mild congenital hemophilia A (HA) and type 2N (Normandy) von Willebrand disease (VWD) (2NVWD), have in common a mild reduction in FVIII:C and normal VWF:Ag and VWF:RCO. HA is X-linked recessive for which FVIII replacement is the treatment of choice, whereas 2NVWD is autosomal recessive for which VWF replacement is the treatment of choice, hence the importance of accurate diagnosis for genetic counseling and treatment. Options include analysis of FVIII binding to VWF (VWF:FVIIIb) or, a more costly option, genotyping.

Aims: Evaluate the performance characteristics of VWF:FVIIIb assay.

Methods: VWF:FVIIIb assay was performed on plasma samples from normal donors ($n = 15$), HA patients (pt) ($n = 30$) or VWD (type 1 $n = 38$; type 2A/B or 2M $n = 17$; type 2N $n = 2$), utilizing the Asserachrom VWF:FVIIIb kit (Stago, Parsippany, NJ, USA) following the manufacturers' instructions. FVIII:C, VWF:Ag, FVIII:C to VWF:Ag ratio and VWF:FVIIIb were analyzed.

Results: Normal donors: FVIII:C: VWF:Ag ratio: mean 1.04 (range: 0.81 to 1.5), VWF:FVIIIb: mean of 103% (standard deviation (SD):7.7%) verifying the package insert mean 104% (SD 10.4%). For type 1 VWD pt, FVIII:C: VWF:Ag ratio: mean 1.83 (range: 0.78 to 2.79), VWF:FVIIIb: mean of 99.1% (standard deviation (SD):12.6%). One type 1 VWD pt had FVIII:C: 31, VWF:Ag 40, VWF:RCO 28, FVIII:C: VWF:Ag ratio: 0.8 and VWF:FVIIIb: of 12% and was heterozygous for the exon 20 (R854Q) mutation. Thus he was compound heterozygous for type 1 VWD and 2N VWD. For HA pt FVIII:C: VWF:Ag ratio: mean 0.2 (range: 0 to 0.5), VWF:FVIIIb: mean of 98.7% (SD:16.8); no pt had reduced VWF:FVIIIb. The type 2N VWD pt had FVIII:C:VWF:Ag ratio of 0.5 and VWF:FVIIIb 7%.

Conclusion: In this cohort, 0% of mild HA pt and 1/38 (2.6%) of type 1 VWD pt had FVIII:VWF binding defect. In type 1 VWD pt, a FVIII:C:VWF:Ag ratio of less than 0.8 may predict a FVIII:VWF binding defect. Additional analysis of performance characteristics is ongoing and will be presented at the meeting.

Disclosure of Interest: None declared.

PO649-MON

New insights into type 3 von Willebrand disease: the type 3 von Willebrand disease international registries and inhibitor prospective study (3WINTERS-IPS) project update

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Background: von Willebrand disease type 3 (VWD3) is of major interest because of severe clinical presentation, need for replacement therapy with VWF/FVIII concentrates and the risk of anti-VWF inhibitors developing after treatment.

Aims: To evaluate in large cohort of VWD patients the relationship between standardised phenotypic, genotypic, clinical data and bleeding tendency, response to therapy with VWF/FVIII concentrates and the risk of anti-VWF inhibitor development.

Methods: 3WINTERS-IPS is a multicenter, European and Iranian observational, retrospective and prospective study on patients with VWD3. Patients meeting the enrolment criteria were enrolled at each participating centre and data entered into the project database.

Results: 251 VWD3 cases are included on the database with a gender distribution 106/145 (M/F); median age 27 (1–75) yr and median bleeding score (BS) 12 (1–33). Median (range) of local lab test were: VWF:Ag 1.9 (<1–7) IU dL⁻¹; FVIII:C 2.3 (<1–15) IU dL⁻¹. Anti-VWF antibodies are reported present in 11. Molecular genetic analysis was undertaken at local sites in 55 patients all from EU sites. Of these, 31 (56%) are compound heterozygous (CH), 19 (35%) are homozygous (H) and 5 (9%) are apparently heterozygous with only one mutation found. In the CH group, there is a full range of mutation types including large deletions, small deletions/insertions, missense and nonsense mutations and splice site changes. One CH case had 2 missense mutations, both resulting in loss of a cysteine (p.C1227R and p.C2283R). Of the H group, 6 (31%) had splice and 5 (26%) had nonsense mutations. Of the 4 H with missense mutations, 2 resulted in loss of cysteines p.C2212R and p.C2362F and 2 had p.N2546Y. One H case had a gene conversion involving p.Q1311*.

Conclusion: This initial data confirms that VWD3 is phenotypically and genotypically heterogeneous.

Disclosure of Interest: None declared.

PO650-MON

Efficacy and safety of von Willebrand factor concentrate with a low FVIII content in severe VWD children less than 6 years of age

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Background: Treatment with a VWF concentrate almost devoid of FVIII (vWF SD-35-DH) has been previously studied in patients of any age with inherited VWD in several clinical trials. A study in children <6 years was specifically designed to further document clinical and biological data.

Aims: The study aimed to evaluate efficacy, safety of this VWF product for the prevention and treatment of hemorrhage or surgical bleeding.

Methods: This multinational, open-label study was approved by Ethics Committees. After signed consent form from parents, children with severe VWD were followed for ≥18 months. For surgery and on-demand therapy, dosing was individualized to achieve a VWF:RCo peak level of 100% if recovery was evaluated or 60–100 IU kg⁻¹ if recovery was not available. Overall efficacy was rated by the investigator using a 4-point scale. For long-term prophylaxis (LTP), forced titration (50 IU kg⁻¹ once per week to 30 IU kg⁻¹ every 2 days) was provided as a guidance to minimize bleeding.

Results: Nine patients (0–5 years) received the study drug: 7 type 3, 1 type 2, 1 type 1, all with VWF:RCo <10%. Mean recovery at 15 min post-infusion was 1.8 IU dL⁻¹ IU⁻¹ kg⁻¹. Of 62 bleeds (median infusion dose 54 IU kg⁻¹, range 44–114), 89% were controlled with 1 (73%) or 2 infusions (16%). A priming FVIII dose was co-administered once. Hemostatic efficacy was rated as excellent/good in 90% of cases. Six patients underwent 11 minor surgeries, managed with a median of 2 infusions (range 1–6) and a dose of 56 IU kg⁻¹ infusion⁻¹ (range 41–104). Hemostasis was rated as excellent in all procedures. The annual bleeding rate was 3.4 for a child treated 4 years by LTP, 3 times/week. No related adverse events were reported over 770 cumulative exposure days and a mean participation of 3.1 years. No inhibitor development or thrombotic event or hypersensitivity reactions were observed. The negative status for the parvovirus B19 at inclusion remained negative.

Conclusion: This VWF product was efficient and well tolerated in severe VWD children <6 years of age.

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PO651-MON

Overdosing of replacement therapy in perioperative von Willebrand patients: a lot 'to win'

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Background: von Willebrand Disease (VWD) is the most common inherited bleeding disorder. VWF/FVIII concentrates are generally administered in moderate or severe VWD patients in case of bleeding or surgical procedures. Dosing is then mainly based on body weight, taking prescribed target values of FVIII and sometimes VWF into account. This is often complex.

Aims: To evaluate current perioperative management of VWD patients in relationship to VWF and FVIII target levels.

Methods: We included 65 VWD patients (historical VWF levels ≤ 0.30 IU mL⁻¹ and/or FVIII ≤ 0.40 IU mL⁻¹) from two Academic Hemophilia Treatment Centers in the Netherlands (median age 50 years, median weight 76 kg), who underwent 99 surgical procedures between 2000–2014. VWF/FVIII concentrate (ratio 2.4:1) was infused based upon FVIII content, targeting VWF and FVIII levels as defined by the National Hemophilia Consensus. We collected type of VWD, patient-, and surgical characteristics and achieved VWF activity (VWF:Act) and FVIII trough levels. The Medical Ethics Committee waived the need for a formal review.

Results: During the first 36 h after surgery median VWF:Act was 1.21 IU mL⁻¹ [IQR:0.85–1.76 IU mL⁻¹] and median FVIII was 1.32 IU mL⁻¹ [IQR:1.04–1.70 IU mL⁻¹]. Most VWF:Act and FVIII levels were above target level of at least 0.80 IU mL⁻¹, respectively 76% (median deviation of 0.71 IU mL⁻¹) and 89% (median deviation of 0.56 IU mL⁻¹). In addition, 24% of VWF:Act levels and 11% of FVIII levels were under this target level. Postoperatively, after 36 h all FVIII levels were above recommended target levels (median FVIII 1.59 IU mL⁻¹ [IQR:1.13–2.04 IU mL⁻¹]). Clinically relevant bleeding occurred in only two surgical procedures. No thrombotic events were observed.

Conclusion: Overdosing of replacement therapy is significant in current perioperative management of VWD patients, using FVIII based dosing. These data support alternative dosing strategies leading to more individualized dosing with an increase in quality of care and possible concomitant cost reduction.

Disclosure of Interest: None declared.

PO652-MON

A microchip flow-chamber system (T-TAS[®]) reflects the clinical phenotypes in patients with type 1 von Willebrand disease

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Background: von Willebrand disease (VWD) represents clinically heterogeneous phenotype. However, von Willebrand factor ristocetin cofactor activity (VWF:RCO) do not always reflect the clinical phenotype, especially in type 1 VWD, under non-physiological static condition. We've recently reported the possibility of clinical application for VWD using a microchip flow chamber system (T-TAS[®]; Fujimori Kogyo).

Aims: We investigated whether T-TAS[®] reflected the clinical phenotype (bleeding score; BS) in type 1 VWD.

Methods: Fifty of type 1 VWD (4 mo–72 y.o. median 22 y.o.) were enrolled (VWF:RCO/Ag; $18.9 \pm 14.0/23.0 \pm 14.6$ U dL⁻¹). Using hirudin- and citrated-added whole blood, microchips coated with either collagen (PL-chip) or collagen/thromboplastin (AR-chip) were used to evaluate platelet thrombus formation (PTF) at 1000 or 2000 s⁻¹ (high shear) and fibrin-rich PTF at 240 s⁻¹ (low shear), respectively. Flow pressure curves were monitored and time until 10 kPa (T₁₀) was evaluated.

Results: PL-T₁₀ and AR-T₁₀ in normal control ($n = 30$) were 3.5 min [CI: 2.4–6.6] and 12.2 min [8.6–16.6], respectively. PL-T₁₀ obtained in type 1 VWD showed greater correlation with BS ($R^2 = 0.45$), reported by Tostetto et al. (JTH 2011), compared to VWF:RCO ($R^2 = 0.35$), irrespective of flow rates. AR-T₁₀ showed the lower correlation ($R^2 = 0.18$), however. In cases with PL-T₁₀ ≤ 10 min (maximal limit for detection) in PL-flow, their VWF:RCO were higher ($22.7 \pm 13.8/9.8 \pm 10.1$ U dL⁻¹, $P < 0.005$) and their BS were much lower ($3.5 \pm 2.9/8.8 \pm 3.5$, $P < 10^{-6}$) than those with undetected PL-T₁₀ (>10 min), indicating significant difference in BS. Cases with PL-T₁₀ ≤ 8 min, particularly, showed most significant difference with BS than those with >8 min (BS; $2.8 \pm 2.1/8.7 \pm 3.3$, $P < 10^{-9}$), supporting the presence of much less cases with discrepancy between PL-T₁₀ and BS.

Conclusion: Evaluation in T-TAS with PL-chip, in particular PL-T₁₀, could be very useful for distinguishing and predicting clinical bleeding phenotype in type 1 VWD.

Disclosure of Interest: None declared.

PO653-MON

The use of next-generation sequencing to screen for disorders of coagulation using a 24 gene panel

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Background: Next-generation sequencing (NGS) is a valuable tool. Recent improvements in accuracy and reductions in cost of NGS and exon capturing platforms have widened the applicability of targeted exon sequencing to NHS molecular diagnostics laboratories, enabling the identification of a broader range of variants to explain the heritability of complex diseases, including haemostatic disorders.

Aims: We sought to investigate the clinical utility of NGS for understanding haemostatic disease by sequencing 24 coagulation genes using a designed TruSeq Custom Amplicon (TSCA) panel.

Methods: Samples were run on the Illumina MiSeq platform. The TSCA panel was designed with 508 amplicons covering 24 genes, including the 14 genes routinely tested by Sanger sequencing in our centre (F8, F9, VWF, F5, F7, F10, F11, F13A1, MYH9, FGA, FGB, FGG, GP1BA and SERPINC1). Ten additional genes linked to coagulation, platelet and thrombotic disorders were included: LMAN1, MCFD2, F13B, ADAMTS13, GP1BB, GP9, ITGA2B, ITGB3, PROC and PROS1.

Results: 133 diagnostic samples were screened in parallel by NGS and Sanger sequencing with 100% concordance. Sequence variants were found in 117 samples (88%). 112 variants were detected of which 47 were novel variants. The novel variants found were, 70% missense, 4% splicing, 20% small deletions, 2% small insertion and 4% duplications. 16 samples were NGS negative, of these 9 had a copy number variation mutation (7 deletions, 2 duplications). 10 samples had additional deleterious variants in genes other than those indicated by phenotype suggesting previously uncharacterised genetic complexity in coagulation disorders.

Conclusion: The 24 NGS gene panel is an effective method for the clinical diagnosis of multigene and single large gene disorders. Variants with functional effects were identified in 88% of our patients. The

NGS panel TAT is compatible with conventional screening and is cost effective. This preliminary study demonstrates the potential advantages of NGS in clinical mutational screening.

Disclosure of Interest: None declared.

PO654-MON

Course of von Willebrand factor (VWF) during pregnancy in women with von Willebrand disease (VWD)

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Background: Von Willebrand Factor (VWF) is known to be elevated in pregnancy. Women with von Willebrand Disease (VWD) are at risk of a variety of complications including postpartum haemorrhage or miscarriage. However, the course of VWF and associated parameters throughout pregnancy in women with VWD are not well-investigated. **Aims:** The aim of this study was to determine the course of VWF and associated parameters in pregnant women with VWD over time.

Methods: In total 34 pregnant patients (mean age: 31.6; 20–46) were included in this study (28 patients with VWD type 1, 4 with type 2A and 2 with type 2M). Blood samples were consecutively collected up to 279 days of pregnancy to analyse FVIII:C, VWF antigen (VWF:Ag) and VWF activity (VWF:GPIbM), additionally at non-pregnant baseline. A total of 109 healthy pregnant women served as a control to establish pregnancy adopted reference ranges.

Results: Our data showed a significantly increase of FVIII:C ($88.2\text{--}200.4\text{ IU dL}^{-1}$) and VWF activity ($71.1\text{--}187.3\text{ IU dL}^{-1}$) during pregnancy to values more than double compared to early pregnancy or baseline. VWF:Ag rose more than threefold ($75.5\text{--}242.2\text{ IU dL}^{-1}$) from first to third trimester. However, mean values remained below the reference range of healthy pregnant women, except for the last month of pregnancy.

Conclusion: Here, we show the natural course of VWF in patients with VWD during pregnancy. Although a clear increase of mean VWF is demonstrable, VWF is still below the lower limit of reference range from beginning until late pregnancy. However, the clinical relevance of this disproportion remains to be clarified.

Disclosure of Interest: None declared.

PO655-MON

Identification of large VWF gene deletions and duplications by MLPA in the south moravian (Czech Republic) VWD cohort in the BRNO-VWD study

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Background: Von Willebrand Disease (VWD) is an autosomally inherited bleeding disorder caused by a quantitative or qualitative defect of von Willebrand factor (VWF). University Hospital Brno (Czech Republic) and Antwerp University Hospital (Belgium) performed a family-based characterization of VWD in the Czech Republic. 201 blood samples were collected from VWD patients representing 96 families. After analysis of genomic DNA by DNA sequencing of the 52 ex-

ons, including intron/exon boundaries, at least 1 probable causal sequence variation was found in 138/201 patients (106/138 with a mutation described in the ISTH VWD database and 32/138 new). In 23/138 a compound heterozygous variation was found. In 64/201 patients (mainly type 1 VWD) no causative mutation was found. VWD can also be caused by multiple heterozygous/homozygous exon deletions or duplications in form. Heterozygous deletions will not be detected by the absence of the PCR product because of the presence of the normal allele.

Aims: To investigate the samples with no causal mutation found by DNA sequencing for large VWF gene deletions/duplications.

Methods: Multiplex ligation-dependent probe amplification (MLPA; MRC Holland) was used to investigate the samples where no mutation ($n = 64$) or a putative new mutation ($n = 32$) was found by direct DNA sequencing.

Results: So far 74/96 samples were investigated. In 14 patients a large heterozygous gene defect was by MLPA: deletion of exons 1–3 ($n = 2$), exon 1–6 ($n = 2$), exon 20 ($n = 1$), exon 28 ($n = 1$), exon 31 ($n = 2$) and a whole allele ($n = 4$), duplication of exon 20 ($n = 2$). All these defects, in patients without a causative mutation, were found in type 1, with exception of an exon 31 deletion which was found in type 2M.

Conclusion: In this VWD cohort around 10% of all VWD type 1 is caused by a large deletion/duplication in the VWF gene, which rises to 15% of all type 1 where no causal mutation was found by DNA sequencing. This makes MLPA essential in the genetic investigation of VWD, in addition to direct DNA sequencing.

Disclosure of Interest: None declared.

PO656-MON

Baseline factor viii plasma levels and age at first bleeding in patients with severe forms of von Willebrand disease

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Background: Von Willebrand disease (VWD) is the most common inherited bleeding disorder. The age of bleeding onset is highly variable, also in patients with similar degree of severity.

Aims: Aim of this study was to evaluate whether baseline factor VIII (FVIII) plasma levels correlate with age at first bleeding in patients with severe forms of VWD.

Methods: One hundred and three patients with severe forms of VWD (VWF:RCO $<6\text{ IU dL}^{-1}$ [6 VWD1, 73 VWD2 and 24 VWD3]) undergoing a medical examination between September 2010 and September 2013 were included. In all patients, the age of bleeding onset was recorded. The relationship between baseline FVIII levels and age at first bleeding was tested using a multivariable linear regression model, adjusting for sex.

Results: The median age at first bleeding was lower in patients with VWD3 than in those with severe VWD1 or VWD2 (1 year vs 7 and 8 years, respectively, $P < 0.0001$). A non-linear relationship between FVIII levels and age at first bleeding was found, the latter increasing by 5 years for every 10 IU dL^{-1} increase of FVIII ($\beta = 4.95$ [95%CI: 2.02–7.87]) until levels of 30 IU dL^{-1} , after which the age increased slowly. In 65 patients (63%) there was more than 6 months of delay between bleeding onset and VWD diagnosis, with no difference over decades.

Conclusion: Baseline FVIII plasma levels influence the age at first bleeding onset in patients with severe forms of VWD. Despite

improvements in the last decades, the diagnosis of VWD is still delayed in a consistent number of patients.

Disclosure of Interest: None Declared.

PO657-MON

Type 1 VWD does not lead to increased gingival bleeding

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Background: Von Willebrand disease (VWD) results in prolonged bleeding time and enhanced mucous bleeding. Mouth bleeding is a frequently reported symptom of VWD evaluated by several VWD bleeding scores. However, gingival bleeding is also known as a leading symptom of plaque-induced gingivitis and untreated periodontal disease.

Aims: This study evaluates whether type 1 VWD determines an increased susceptibility to gingival bleeding.

Methods: In this case-control study, fifty cases and forty controls were examined periodontally with respect to gingival bleeding (Gingival Bleeding Index [GBI]), bleeding on probing (BOP), supragingival bacterial deposits (Plaque Control Record [PCR]), further periodontal variables (periodontal inflamed surface area [PISA], vertical probing attachment level) and received a professional tooth cleaning. Type 1 VWD patients were in long-term treatment of the haemophilia centre and the controls were matched according to sex, age, number of teeth and smoking. In the control group a bleeding disorder had been excluded.

Results: Patients with VWD (median age 42.1 ± 13.7 years, 86% female) reported more frequently periodontal symptoms, such as bleeding (34% vs. 20%) or swelling (54% vs. 32.5%) and their teeth were less frequently professionally cleaned (58% vs. 77.5%, $P = 0.051$). The gingival bleeding index, however, was higher in controls (12.2%) than in VWD (10%) and was similar regarding BOP between VWD (17%) and controls (17.2%). Cases and controls were well balanced regarding periodontal control variables indicating similar degree of bacterial deposits. In mild type 1 VWD periodontal examination and professional tooth cleaning were carried out without increased risk of bleeding.

Conclusion: Gingival bleeding in VWD patients may be triggered by gingival inflammation and is not a genuine symptom of VWD.

Disclosure of Interest: None declared.

PO658-MON

Joint surgery in moderate and severe von willebrand disease: a cross-sectional study.

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Background: Joint bleeds are reported by 23% of moderate and severe VWD patients and might be associated with orthopaedic surgery.

Aims: To assess the prevalence, indications, management and complications of joint surgery in VWD patients.

Methods: In the Willebrand in the Netherlands study 804 moderate and severe VWD patients (VWF activity and/or antigen ≤ 30 U dL⁻¹) completed a questionnaire on joint bleeds, joint damage and orthopaedic surgery. We additionally retrieved medical file data on patients with shoulder, elbow, hip, knee or ankle surgery.

Results: 14% (116/804) of the VWD patients self-reported large joint surgery. These patients reported joint bleeds more often compared to VWD patients not reporting this surgery (41% vs 20%, $P < 0.001$) and more often large joint damage (61% vs. 20%, $P < 0.001$). We obtained medical file data on 126 large joint surgeries in 79 VWD patients, mostly knee (47%), followed by ankle (19%) and hip (18%) surgery. Of the procedures 44% (55/126) was arthroscopic. Most operations (76/126, 60%) took place in haemophilia treatment centres (HTC). Surgery was performed because of joint damage due to prior joint bleeds in 25% (26/108). Preoperative clotting factor correction (CFC) to prevent bleeding was administered in most cases (88/109, 81%). Still, post-operative bleeding occurred in 23/126 surgeries (18%) with no significant difference between arthroscopic surgery compared to arthrotomy (27% vs. 18%, $P = 0.31$), preoperative CFC use (17% bleeding in procedures with CFC vs. 33% without CFC, $P = 0.13$), nor between HTC vs. non HTC (17% vs. 20%, $P = 0.68$). Bleeding complications occurred more often in surgeries without monitoring of FVIII or VWF level (10/37 vs. 3/39, $P = 0.025$).

Conclusion: Large joint surgery is reported by 14% of moderate and severe VWD patients, related to joint bleeds in 25% of the procedures and frequently associated with bleeding complications despite prophylactic CFC treatment. Monitoring of FVIII or VWF levels around orthopaedic surgery may result in less bleeding complications.

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PO659-MON

Ethnic and racial variation of plasma VWF in healthy controls

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Background: Prior work from ZPMCB-VWD demonstrated higher VWF levels in African American (AA) healthy controls (HC), we now focus on Hispanic (HA) and Asian Americans (AsA).

Aims: To identify ethnic variation in plasma VWF levels and the impact of ABO blood group in HC.

Methods: ZPMCB-VWD has enrolled 3217 subjects including 444 HC [130 Caucasian American (CA), 193 AA, 46 HA, 52 AsA, and 23 other/multiethnic] who had full-length VWF sequencing, ISTH bleeding score (ISTH-BAT), ABO blood-type, and VWF assays: VWF:Ag, VWF:RCO, VWF:GPIbR, VWF:GPIbM, VWFpp, VWF:CB3, and VWF:CB4. Ethnicity was self reported and multiple ethnicities were excluded. Geometric mean (GM) and two geometric standard deviation range (2GSD) were determined and results compared.

Results: In 444 HC, 11 had borderline positive BAT, but none had reduced VWF:Ag or VWF:RCO. 14 HC had VWF:Ag levels < 50 IU dL⁻¹ but none of these had abnormal BAT. VWF:Ag (IU dL⁻¹) GM and 2GSD were 116 (57–237) for CA, 124 (53–289) for AA, 86 (55–165) for HA, and 86 (39–192) for AsA. Both HA and AsA groups were significantly reduced and AA elevated compared to CA ($P < 0.0001$). VWF:RCO/VWF:Ag ratio was 1.04 ± 0.18 for CA,

0.80 ± 17 for AA ($P < 0.0001$), 0.94 ± 0.16 for HA ($P < 0.0001$), and 0.93 ± 0.14 ($P < 0.0001$) in AsA. In all cohorts type O individuals had significantly lower VWF:Ag with the most striking difference in AsA with mean VWF:Ag of 66 in blood group O and 109 in non-O. Of note, 8 of 20 type O AsA had VWF:Ag <55 yet none had abnormal BAT. In HA, 30.4% had nonsynonymous sequence variations (SV) with 10 having novel or rare SV. 2 of the HA subjects had VWF:Ag <50 IU dL⁻¹ with A631V and P1162L that are probably damaging by PolyPhen (0.975). In AsA, 14% have SV with 9 having novel or rare SV. Of the 6 AsA subjects who had VWF:Ag <50 IU dL⁻¹, an R273W and a new C799W were identified and probably damaging (0.99–1.00).

Conclusion: Hispanic Americans and Asian Americans have lower levels of VWF than Caucasian, but have abnormal VWF:RCo/VWF:Ag ratios and some unique VWF sequence variations.

Disclosure of Interest: None Declared.

PO660-MON

A comparative study of microfluidic flow assays and clinical assays in type I von Willebrand's disease

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Background: Predicting bleeding in type I von Willebrand disease (VWD) remains a challenge using conventional assays. VWF and platelet function and are shear stress-dependent, but of the available assays, only the PFA-100 incorporates shear stress. Here, we compare the sensitivity to VWF levels of microfluidic flow assay (MFA) to conventional clinical assays.

Aims: Compare the sensitivity of the MFA, PFA-100, and ristocetin-induced aggregation to VWF antigen levels in type I VWD.

Methods: Whole blood from individuals ($n = 22$) with suspected type I VWD was collected into sodium citrate. Recalcified blood was perfused over a 150 μ m strip of type I collagen through microfluidic channels ($h = 50$ μ m, $w = 500$ μ m) at wall shear rates of 150, 750, and 1500 s⁻¹ for 5 min. Transient platelet accumulation was recorded by relief-contrast imaging and quantified by three metrics; (i) lag time (time to 5% coverage), (ii) velocity (linear growth phase), and (iii) clot volume. PFA-100 closure time, platelet aggregation (VWF:RCo), VWF antigen levels (VWF:Ag), and bleeding assessment tool (BAT) were collected for each sample.

Results: VWF:Ag varied between 5 and 106 in the cohort, with 11 patients exhibiting levels below 50. Both the PFA-100 and the MFA showed dramatic loss in platelet function VWF:Ag <40 . The lag time for the 750 s⁻¹ shear condition was sensitive to VWF:Ag (correlation coefficient (CC) = -0.84 , $R^2 = 0.72$), with the 150 s⁻¹ condition only affected for VWF:Ag <10 (CC=0.21). There was little adhesion at 1500 s⁻¹ for VWF:Ag <40 . PFA-100 was insensitive to VWF:Ag <40 as it reached maximum closure time. VWF:RCo lacked sensitivity to VWF:Ag <40 (CC=0.61). Since eight of our eleven patients were under the age of 18, the BAT only correlated with age.

Conclusion: These data suggest that platelet accumulation on collagen at a shear rate of 750 s⁻¹ is more sensitive to mild type I VWD than the PFA-100 and VWF:RCo and may provide a clinical marker for bleeding in severe to mild type I VWD.

Disclosure of Interest: None declared.

PO661-MON

Total thrombus-formation analysis system (T-TAS) for the monitoring of von Willebrand factor-FVIII concentrate treatment in patients with von Willebrand disease type 3

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Background: Patients with von Willebrand disease type 3 (VWD-3) often need prophylactic medication with VWF-FVIII concentrates. Common global hemostatic tests like rotational thromboelastometry (ROTEM[®]) are not sensitive for monitoring of VWF

Aims: To study the effect of VWF-FVIII concentrate (Haemate[®]) in patients with VWD-3 with a new global hemostatic method, the Total Thrombus-formation Analysis System (T-TAS[®]), which measures in whole blood under flow conditions.

Methods: Ten patients with VWD-3 were monitored before and 30 min after their usual prophylactic dose of Haemate[®]. T-TAS[®] was compared to the levels of VWF, FVIII and to ROTEM[®]. The atheroma (AR)-chip coated with tissue factor and collagen measures under venous flow conditions and the platelet (PL)-chip coated with collagen under arterial flow conditions. The AUC (area under the flow pressure curve) shows the strength of thrombus formation at capillary pressures of 10 and 30 kPa, respectively.

Results: The patients (median age 49 (IQR 28–76) years) received a median dose of 27 (IQR 15–35) U of Haemate[®]. One of them had an ongoing gastro-intestinal bleed and was treated also the day before while the other 9 had received Haemate[®] prophylactically 2–7 days before.

In the 9 patients with no bleeding the values before and after treatment were; VWF 0.10 (0.04–0.26) vs 0.85 (0.28–1.22) kIE L⁻¹ ($P < 0.01$) and FVIII 0.24 (0.02–1.40) vs 1.01 (0.32–2.1) kIE L⁻¹ ($P < 0.01$). ROTEM[®] showed no significant changes after treatment. T-TAS variables; AR-chip; AUC30 385 (39–528) vs 1013 (103–1459) ($P < 0.01$) and PL-chip AUC10 7 (3–32) vs 25 (5–57) ($P = 0.01$). The patient with a bleed had almost normal VWF; 0.48 before and 1.54 kIE L⁻¹ after. The AUC:s increased with both T-TAS chips. The ROTEM[®] clotting time was prolonged and the maximum clot firmness increased in this patient.

Conclusion: T-TAS can detect therapeutic effects of VWF-FVIII concentrate, and can give rapid information about the coagulation profile in whole blood in VWD-3 patients.

Disclosure of Interest: A. Ågren: None Declared, P. Hjemdahl Grant/Research Support from: Fujimori Kogyo Co., Ltd, M. Blombäck: None Declared, K. Hosokawa Employee of: FUJIMORI KOGYO CO, LTD, M. Holmström: None Declared.

PO662-MON

Surveillance study of safety and efficacy of a VWF/FVIII concentrate in patients with von Willebrand disease (WIL-20) – a planned interim analysis

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Background: Non-interventional studies are a valuable tool to provide real-life information about VWD treatment.

Aims: To confirm data from previous clinical studies and to ensure the long-term consistency of use of a VWF/FVIII concentrate in a 1:1 ratio in routine clinical practice.

Methods: This non-interventional, post-marketing study in VWD was designed to document the safety, tolerability, and efficacy in the prophylaxis and treatment of bleeding, including surgeries. Treatment regimen and dosages were at the discretion of the physicians. Main endpoints were tolerability (3-point verbal rating scale) and the incidence of reported ADRs. Thrombogenicity and immunogenicity were optional. The efficacy in on-demand treatment and surgeries was assessed using 4-point haemostatic efficacy scales, for prophylaxis it was based on the frequency of breakthrough bleeds.

Results: The interim analysis comprises 63 patients in the safety population, 10 patients in the on-demand, 18 patients in the prophylaxis and 33 patients in the surgery population. Thirty-nine bleeding episodes (BEs) were treated in 10 patients with 100% excellent/good efficacy.

Mean annualised bleeding rate in the 11 out of 18 patients, who received continuous prophylaxis was 3.50 ± 6.0 (median 1.9; range 0–20.8) for spontaneous bleeds and 6.23 ± 7.16 (median 3.77; range 0–20.8) for all types of bleeds.

Nineteen major and 23 minor surgeries were performed in 33 patients. Efficacy was rated excellent/good in 41 (98%), and moderate in 1 surgery (2%).

Tolerability was assessed in 2244 and rated excellent for 2099 infusions (93%). No neutralising VWF inhibitors and no thrombotic events were reported. Only three related ADRs were documented (hypersensitivity like reactions).

Conclusion: The data of this planned interim analysis confirm the safety, tolerability and efficacy of the VWF/FVIII concentrate in on-demand treatment of BEs, prophylaxis, and the prevention of excessive bleeding during surgeries in patients with VWD, under routine clinical conditions.

Disclosure of Interest: K. Khair Speaker Bureau of: Octapharma AG, M. S. Cruz: None Declared, S. Werner Employee of: Octapharma Inc, S. Knaub Employee of: Octapharma AG, A. Iorio Consultant for: Octapharma AG.

PO663-MON

Predictors of complete response to DDAVP in patients with von Willebrand disease

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Background: Desmopressin (DDAVP) stimulates the release of von Willebrand factor (VWF) and factor VIII (FVIII) from endothelial cells and platelets, increasing their levels in plasma. It can be used to treat or prevent bleeding in patients with von Willebrand disease (VWD).

Aims: To develop a predictive model of complete response to DDAVP in patients with VWD.

Methods: Data of 168 type 1 or 2 VWD patients (median age 35 y [IQR:24–48], 57% females) tested for response to DDAVP in our Center were recorded. VWF (RCo and Ag) and FVIII plasma levels were measured before and 1 h, 2 h and (in 127 patients) 4 h after $0.3 \mu\text{g kg}^{-1}$ DDAVP infusion. Complete response to DDAVP was defined as both VWF:RCo and FVIII:C $\geq 50 \text{ IU dL}^{-1}$ after DDAVP infusion. Two logistic regression models (for response at 1 h and 4 h) were fitted, containing sex, age, baseline plasma and intraplatelet VWF:RCo, ABO blood group, and (only for response at 4 h) VWF propeptide to Ag ratio (pp/Ag) as predictors. Predictive ability of the logistic models was measured as the area under the ROC curve (AUC).

Results: 115/168 patients (69%) had complete response at 1 h and 63/124 (51%) at 4 h. The best predictors of response at 1 h were baseline VWF:RCo (adjusted odds ratio 4.73 [95%CI:1.93–11.58] for every 5 IU dL^{-1} increase) and intraplatelet VWF:RCo (2.49 [95%CI:1.33–4.66] for every 0.05 IU dL^{-1} increase). At 4 h, the best predictors were baseline VWF:RCo (7.25 [95%CI:1.82–28.84] for every 5 IU dL^{-1} increase) and pp/Ag, which showed a bimodal relationship, with odds of response decreasing for values >2.90 , likely because of a rapid VWF clearance in patients with high pp/Ag. After 200 bootstrap replicates, the AUCs of the models for response at 1 h and 4 h were 0.96 and 0.94, respectively.

Conclusion: Complete response to DDAVP in patients with VWD was mostly predicted by baseline plasma and intraplatelet VWF:RCo (at 1 h) and by baseline plasma VWF:RCo and pp/Ag (at 4 h). Both predictive models have a very good discriminative ability.

Disclosure of Interest: None declared.

PO664-MON

Major surgeries in children and adults with von Willebrand disease managed with a high-purity human VWF/FVIII concentrate in a physiological 1:1 ratio

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Background: Major surgeries in patients with VWD can be associated with life-threatening risk of excessive bleeding, thus prophylactic treatment with exogenous VWF/FVIII may be required.

Aims: To evaluate the overall haemostatic efficacy of a VWF/FVIII concentrate in a physiological 1:1 activity ratio in preventing excessive surgical bleeding in children and adults with VWD, a prospective, open-label, multi-center, phase III clinical study was conducted. Results for major surgeries are presented here.

Methods: IVR was investigated in all patients prior surgery for recommending individual dosing, which was to achieve VWF:RCo peaks of 100% and to maintain trough levels around 50% until wound healing. Actual dosing was at the discretion of the physician. VWF:RCo, VWF:Ag, and FVIII:C levels were collected throughout the study. Haemostatic efficacy was assessed independently by surgeons and haematologists, using objective 4-point ordinal efficacy scales. Assessments were adjudicated by an independent data monitoring committee. Safety and immunogenicity were monitored throughout.

Results: Twenty-one major surgeries in 20 patients were treated in the study. The majority (17) were VWD type 3, 3 were VWD type 1 and 1 VWD type 2. Mean loading dose (VWF:RCo) given was 55.5 IU kg^{-1}

with a mean maintenance dose of 30 IU kg⁻¹. Mean FVIII:C and VWF:RCo peak levels for each patient during maintenance infusions (day 1–7 post surgery) ranged from 120–145%, and 66–98%, respectively, with no accumulation of FVIII:C. 95.2% of major surgeries managed with the 1:1 VWF/FVIII concentrate had efficacy rated as excellent/good. There was no excessive or uncontrolled intra- or post-operative bleeding or requirement for an alternate VWF/FVIII concentrate. No FVIII:C accumulation or thrombotic events were reported. No neutralizing VWF/FVIII inhibitors or study drug-related serious adverse events were observed.

Conclusion: This data confirms the safety and efficacy of the 1:1 VWF/FVIII concentrate for the management of major surgeries in VWD patients.

Disclosure of Interest: A. Srivastava: None Declared, S. Werner Employee of: Octapharma Inc., M. Serban: None Declared, B. Schwartz Employee of: Octapharma Inc., S. Knaub Employee of: Octapharma AG, C. Kessler Consultant for: Octapharma AG, Speaker Bureau of: Octapharma AG.

PO665-MON

The influence of PAI-1 levels on bleeding phenotype in moderate and severe von Willebrand disease

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Background: Von Willebrand disease (VWD) is the most common inherited bleeding disorder and is characterized by mucocutaneous bleeding. In patients with VWD large variations in bleeding tendency are observed, which are not completely related to VWF levels. To date, the cause of this variability remains unexplained. Studies have described low Plasminogen Activator Inhibitor-1 (PAI-1) activity, an important inhibitor of fibrinolysis, as a minor causative factor in patients with a bleeding tendency. We hypothesized that low PAI-1 levels, may result in a higher bleeding tendency in VWD.

Aims: To investigate whether PAI-1 levels are associated with bleeding episodes of VWD during life.

Methods: PAI-1 levels were measured in plasma from 638 patients with moderate or severe VWD who participated in the 'Willebrand in the Netherlands' (WiN) study, a nationwide multicenter cross-sectional study, using the TECHNOZYM PAI-1 antigen ELISA reagent kit (Technoclone, Vienna, Austria). Bleeding severity was assessed using the Tosetto Bleeding Score (BS-total), which included twelve bleeding items. For additional analysis, also a mucocutaneous bleeding score (BS-mucocutaneous) was used, which included six bleeding items (epistaxis, bleeding from minor wounds, oral cavity bleeding, gastrointestinal bleeding, bleeding after tooth extraction and menorrhagia). Medical ethics committee approval and informed consent were obtained.

Results: PAI-1 levels were significantly higher in men compared to woman [23.3 (IQR 11.7–59.7) vs. 18.3 (IQR 9.4–40.0) ng mL⁻¹, $P = 0.007$]. PAI-1 levels increased with age [Spearman's rho 0.208, $P < 0.001$]. No association was observed between BS-total or BS-mucocutaneous and PAI-1 levels after adjustment for age, gender, VWF:Act and FVIII.

Conclusion: Our study shows that PAI-1 levels do not influence the bleeding phenotype in VWD patients.

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PO666-MON

Dynamic thrombus formation in the whole blood under the shear stress rapidly reflects clinical severity in type 2N von Willebrand disease

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Background: Type 2N von Willebrand disease (VWD) is essentially caused by the impairment of FVIII-binding with VWF, resulting in the discrepancy between FVIII level and VWF level (VWF:Ag and VWF:Rco) in the plasma. Type 2N VWD patients reveal various clinical severity, however, FVIII:C, VWF:Ag or VWF:Rco measured by the plasma-based static assays may not account for their severity of phenotype.

Aims: We investigated the dynamic thrombus formation under high shear stress in the whole blood collected from type 2N VWD patients with severe (Case 1) or mild (Case 2 – 4) phenotype utilizing a flow chamber system, T-TAS[®] (Fujimori Kogyo, Co.) in order to evaluate their hemostatic potentials.

Methods: Whole blood sample containing hirudin were perfused over a microchip coated with collagen at a shear rate of 1000s⁻¹, and the platelet thrombus formation was quantified by monitoring the flow pressure. Parameters, T₁₀ (time to 10 kPa of the flow pressure) and AUC₁₀ (area under curve for 10mins from initiation) were evaluated in Case 1 (female, FVIII:C 5 IU dL⁻¹, VWF:Rco 32%, VWF:Ag 45%) suffering from repetitive metrorrhagia and menorrhagia (Bleeding Score (BS) 27, Bowman M, JTH 2009), Case 2 (female, 8 IU dL⁻¹, 79%, 79%) experiencing only one menorrhagia (BS 2), Case 3 (boy, 4 IU dL⁻¹, 53%, 52%) and Case 4 (male, 4 IU dL⁻¹, 46%, 60%) having no hemorrhagic event (BS 0).

Results: No thrombus formation was observed during perfusion and T₁₀ was apparently prolonged over 10 min in Case 1, whilst thrombus grew up and the capillary occlusion was accomplished in Case 2 (T₁₀ 2.8 min, AUC₁₀ 297 min⁻¹), Case 3 (3.3 min, 321 min⁻¹) and Case 4 (3.2 min, 345 min⁻¹). Administration of VWF/FVIII concentrate improved T₁₀ (7.0 min) and AUC₁₀ (36.4 min⁻¹) in Case 1, and desmopressin further enhanced the thrombus formation in Case 2 (T₁₀ 2.3 min, AUC₁₀ 352 min⁻¹) and 3 (1.8 min, 427 min⁻¹).

Conclusion: Dynamic thrombus formation in the whole blood reflected clinical phenotype of type 2N VWD and it is useful tool monitoring of hemostatic treatment.

Disclosure of Interest: None declared.

PO667-MON

Functional characterisation of a novel splice mutation, c.7887 + 2T>A, associated with type 1 von Willebrand disease

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Background: Mutations causing qualitative or quantitative deficiency of von Willebrand factor (VWF) result in von Willebrand disease (VWD), the most prevalent inherited bleeding disorder. Splice mutations are commonly associated with a mild (type 1 VWD; VWD1) or severe (type 3 VWD) quantitative deficiency because they result in a null VWF allele.

Aims: To investigate the disease mechanism associated with a novel splice mutation, c.7887 + 2T>A, correlated with moderate VWF reduction in a heterozygous VWD1 patient and severe reduction in a compound heterozygous VWD1 patient.

Methods: Due to a lack of patient RNA bacterial exon trap plasmid pET01 was used to assess *in vitro* effect of c.7887 + 2T>A on VWF splicing. Bacterial pCI-neo plasmids expressing either wild-type VWF cDNA (WT) or predicted mutant cDNA were transfected or co-transfected into HEK293T cells and quantity of mutant VWF expressed relative to WT only was assessed using ELISA. Multimer analysis of secreted VWF was performed via electrophoresis on 1.6% (w/v) SDS-agarose gels. High-resolution microscopy of transfected HEK293 cells was performed to assess pseudo-Weibel-Palade body (WPB) formation.

Results: c.7887 + 2T>A was predicted to cause complete skipping of VWF exon 47 resulting in in-frame deletion p.Pro2591_Leu2629del. Expression of the deletion mutant caused significant reduction in VWF secretion compared to WT in both the homozygous (Hom; 58%) and heterozygous (51%) state, but with no significant effect on intracellular retention. In addition, expressed multimers were normal and microscopy highlighted no observable differences in WPB-like structures compared to WT following Hom expression.

Conclusion: c.7887 + 2T>A causes an in-frame deletion of VWF exon 47 resulting in a viable allele that when expressed causes reduced VWF secretion but normal multimer and WPB formation, correlating with disease phenotype. Functional characterisation of splice mutations is important to fully understand their impact on VWF and their mode of pathogenicity.

Disclosure of Interest: None declared.

PO668-MON

Dynamic thrombus formation in the whole blood under the shear stress rapidly reflects the hemostatic potential(s) of type 2A von Willebrand disease unevaluable by plasma-based static assay

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Background: Type 2A von Willebrand disease (VWD) is caused by the qualitative loss of von Willebrand factor (VWF) in the plasma, however, VWF level (VWF:RCO/Ag) measured by the plasma-based static assay appears to be instable and may not evaluate their hemostatic potential.

Aims: We investigated the dynamic thrombus formation under high shear stress with a flow chamber system, T-TAS[®] (Fujimori Kogyo) to evaluate the hemostatic potentials in type 2A VWD.

Methods: Four type 2A VWD (FVIII:C 46 IU dL⁻¹ [CI:23.6–67.8], VWF:RCO 7.2% [1.6–9.6], VWF:Ag 31% [15.6–39.0]), three type 1 as controls (16 IU dL⁻¹ [6.8–50.0], 14% [11–23], 14% [6.4–16]) and an unclassified (type 1 or 2A) case (41 IU dL⁻¹, 1.6–28%, 12–25%) were studied. Whole blood samples containing hirudin were perfused over a microchip coated with collagen at a shear rate of 1000 s⁻¹, and the platelet thrombus formation was quantified by monitoring the flow pressure. Parameters, T₁₀ (time to 10 kPa of the flow pressure) and AUC₁₀ (area under curve for 10 min from initiation) were evaluated.

Results: Thrombus formation was delayed and reduced in type 1 (T₁₀ 7.3 min, AUC₁₀ 51 min⁻¹), whilst no thrombus formation was observed during perfusion and T₁₀ was prolonged over 10 min in all type 2A. Administration of desmopressin (DDAVP) increased VWF and FVIII levels in type 1 plasmas to the maximum (70 IU dL⁻¹ [43–225], 53% [45–111], 40% [32–80]) at 30 min and returned to the baseline within 6–24 h, consistent with the thrombus growth resulting in the capillary occlusion (T₁₀ 3.3 min [1.9–4.0], AUC₁₀ 329 min⁻¹ [235–393]) observed at the time from 30 min to 6 hr after administration. In an unclassified case, no thrombus formation was observed (T₁₀ over 10 min, AUC₁₀ 0 min⁻¹), and DDAVP increased FVIII:C (104 IU dL⁻¹) and VWF:Ag (35%), whilst VWF:RCO, T₁₀ and AUC₁₀ were unaffected, showing type 2A pattern.

Conclusion: Dynamic thrombus formation in the whole blood rapidly differentiated type 1 and 2A VWD indistinguishable by plasma-based static assay and is simple and useful tool for diagnosis.

Disclosure of Interest: None declared.

PO669-MON

Co-occurrence of V.Willebrand disease and bernard soulier-syndrome

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Background: Bernard-Soulier syndrome (BSS) is characterized by dysfunctional or missing platelet receptor for v.Willebrand factor (vWF), low platelet count, large platelets, and massively impaired platelet aggregation in response to ristocetin. v.Willebrand disease (vWD) results in a similar bleeding phenotype, but with normal platelets.

Aims: Marked thrombocytopenia and strongly impaired platelet function were observed in a 35 year old female with vWD. Clinical signs included easy bruising, nosebleeds, and prolonged bleeding after minor injuries since childhood. The patient had been treated several times with corticosteroids and intravenous immunoglobulins in the assumption of immune thrombocytopenia, without any effect on platelet count.

Methods: Coagulation tests, platelet function analyses, and flow cytometry were performed by standard procedures, molecular analyses by next generation sequencing.

Results: vWF antigen was 32% of normal, ristocetin cofactor activity was 23%, Factor VIII activity was 58%. Platelet count was in the range of 40–60 nL⁻¹, mean platelet volume was >18 fl (normal range: 8.8 ± 1.5 fl). Flow cytometry revealed low levels of GP Ib (CD42b) and Gp IX (CD42a) on the platelet surface. No platelet aggregation was observed in response to ristocetin in conventional and whole blood aggregometry. Platelet function analysis using the PFA-100 instrument did not reveal any measurable closure time. Molecular genetic analysis revealed a previously unknown heterozygous mutation c.8251C>T, p.Gln2751X in exon 51 of the vWF gene. A mutation in

this position leads to an abnormal stop-codon with a truncated vWF molecule and defective multimer formation. Analysis of the GP IX gene showed a heterozygous mutation c.182A>G, p.Asn61Ser in exon 3, which has been described by other authors as cause of BSS.

Conclusion: The present case is the first description of co-occurrence of BSS and vWD, as well as the first description of the c.8251C>T, p.Gln2751X mutation of the vWF gene.

Disclosure of Interest: None declared.

Von Willebrand Factor I

PO670-MON

Identification and characterization of aberrant splicing from a novel branch site mutation in von Willebrand factor

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Background: von Willebrand factor (VWF) splicing is disrupted in 15% of von Willebrand disease (VWD) mutations causing aberrant variations of VWF or degradation of the aberrant mRNA. Though most are in highly conserved consensus splice sites, mutations outside of these regions have been linked to splicing defects.

Aims: This study aims to characterize the pathogenic effects of once such mutation, c.6599-20A>T, found in a Type 1 VWD family.

Methods: Peripheral blood samples were taken for DNA, plasma, and blood outgrowth endothelial cell (BOEC) isolation. *In silico* analysis predicted the mutation outcomes and aberrant transcripts were identified. Mutant VWF expression and trafficking were evaluated in BOEC and transfected HEK293T cells and plasma and recombinant VWF were evaluated for functionality.

Results: *In silico* analyses predicted c.6599-20A>T may abolish an acceptor splice site. The mutation segregated with affected family members and patients had similar Type 1 VWD phenotypes. BOEC showed reduced VWF in media ($P < 0.05$) and cell lysates ($P < 0.01$). The BOEC VWF localized to rounded organelles which responded to PMA ($P < 0.05$). Exon 38 skipping was identified introducing a premature termination codon and homozygous expression in HEK293T cells showed mutant secretion decreased ($P < 0.001$) to $2 \pm 0.04\%$ of wildtype and no response to PMA. Lysate VWF was reduced ($P < 0.05$) though not subject to proteasomal degradation. Transfected HEK293 cells showed mutant VWF was diffuse and localized to the endoplasmic reticulum (ER) and lysosome. Heterozygous VWF expression was reduced ($P < 0.05$) to $58 \pm 0.16\%$ with a mild response to PMA. VWF skipping exon 38 has decreased platelet ($P < 0.05$), FVIII ($P < 0.05$), and collagen ($P < 0.01$) binding affinity.

Conclusion: VWF lacking exon 38 would undergo RNA degradation; any VWF produced would be retained in the ER before lysosomal degradation. Minimal mutant:wildtype interaction explains the mild VWD and normal multimers in this family, and their positive response to DDAVP *in vivo* and PMA *in vitro*.

Disclosure of Interest: L. Hawke: None Declared, G. Rivard: None Declared, P. James Consultant for: CSL Behring, Bayer, and Octapharma

PO671-MON

Functional link between von Willebrand factor (VWF) and tissue factor (TF) in flow-dependent thrombus formation: augmentation of intra-thrombus fibrin generation by cathepsin g on VWF-TF complex

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Background: von Willebrand factor (VWF) plays a crucial role on platelet adhesion/aggregation at vessel injury sites under high shear flow conditions. Such platelet aggregates are concurrently reinforced by fibrin generation triggered by tissue factor (TF). However, little is known about the functional link between VWF and TF in flow-dependent thrombus formation.

Aims: To address this issue, we have studied the relevant role of TF on von Willebrand factor (VWF)-dependent thrombus formation by use of an *in vitro* perfusion chamber system.

Methods: Recombinant human TF were co-coated with VWF onto a glass plate to prepare 'surface-immobilized VWF/TF complex'. Recalcified citrated whole blood was perfused over a surface-immobilized VWF/TF complex. In some experiments, neutrophil Cathepsin G (12 ug mL^{-1}) was added to sample blood. Platelet adhesion/aggregation were evaluated by the surface coverage of thrombi. The mural thrombi formed on VWF-surface was double-stained with fluorescently labeled anti-fibrin and anti-fibrinogen antibodies. Fibrin generation was evaluated as a ratio of fibrin relative to fibrinogen fluorescence within mural thrombi.

Results: Surface-immobilized TF significantly augmented flow-dependent fibrin generation as a function of increasing surface density of TF under both low and high shear rate conditions. In addition, immobilized TF significantly up-regulated VWF-dependent platelet adhesion and aggregation under high shear rate conditions. Cathepsin G significantly increased intra-thrombus fibrin generation on immobilized VWF-TF complex under both high and low shear rate conditions.

Conclusion: Surface-immobilized TF and VWF could build mural thrombi in a concerted manner under high shear rate conditions. The present experimental approach revealed that Cathepsin G enhanced the TF-dependent fibrin generation, suggesting the critical involvement of inflammatory responses by leukocytes on thrombus formation under whole blood flow conditions.

Disclosure of Interest: None declared.

PO672-MON

Thrombin-dependent incorporation of von Willebrand factor into a fibrin network

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Background: Under high shear stress Von Willebrand Factor recruits platelets into a growing thrombus via interaction with GPIIb.

Aims: The aim was to investigate the mechanism of VWF incorporation into a fibrin network.

Methods: We monitored the interactions of von Willebrand Factor with fibrinogen, fibrin monomers and polymerized fibrin using different surface techniques and enzymes in purified test system at arterial shear stress.

Results: Our results showed that VWF does not bind to a fibrinogen monolayer or to a polymerized fibrin layer. However, we found proof for binding of VWF to a fibrin monomer layer during the process of fibrinogen-to-fibrin conversion in the presence of thrombin. Using

either Arvin or protease III from snake venom *Crotalus atrox* we showed that VWF interacts with E-domain of fibrin monomers where FpA and/or FpB were situated and this binding is independent of FXIIIa. Additionally we demonstrated that Tyr-2483 and Tyr-2631 located in VWC3 and VWC5 domains respectively, are important for the interaction of VWF with fibrin monomers. These amino acids are well conserved in the VWF sequence from human to zebrafish. Furthermore, under high shear conditions, we were able to show that platelets adhere to fibrin only if VWF had been incorporated. Addition of a GPIb blocking antibody almost completely abolished the adhesion of platelets to the fibrin monomers surface with bound VWF at high shear stress. In the presence of abciximab we observed lower binding of platelets suggesting that both GPIb and GPIIb/IIIa are important for adhesion of platelets.

Conclusion: Our findings demonstrate that the C-domains of VWF and the E-domain of fibrin monomers are involved in the incorporation of VWF during the fibrin polymerization in the presence of thrombin and that this incorporation fosters binding and activation of platelets. Our findings help to elucidate the mechanism of thrombus growth and platelet adhesion under conditions of arterial shear rate.

Disclosure of Interest: None declared.

PO673-MON

MIR-24 regulates von Willebrand factor in diabetes

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Background: An elevated level of Von Willebrand Factor (VWF) in diabetes mellitus patients is associated with increased risk of thrombotic cardiovascular events. The underlying mechanism for how VWF expression is upregulated in diabetes mellitus is poorly understood. MicroRNAs (miRNAs) are small 19–23 nucleotide RNA molecules that negatively regulate the translation of their target mRNAs.

Aims: We aim to determine whether and how VWF is regulated by miRNA.

Methods: A combination of extensive of miRNA screening was applied, followed by analysis in human and mouse samples (diabetes mellitus vs. non-diabetic controls) using both chemical and genetic approaches.

Results: We found that hyperglycemia-induced repression of microRNA-24 (miR-24) increases VWF expression and secretion in diabetes mellitus. In diabetic patients and diabetic mouse models (STZ/high-fat-diet induced and db/db mice), miR-24 is reduced in both tissues and plasma. Knock-down of miR-24 in mice led to increased VWF mRNA and protein levels, and enhanced platelet tethering (spontaneous thrombosis). miR-24 tightly controls VWF levels through pleiotropic effects, including direct binding to the 3'UTR of VWF and targeting furin and the histamine H1 receptor, known regulators of VWF processing and secretion in endothelial cells (ECs). We present a novel mechanism for miR-24 down-regulation through hyperglycemia-induced activation of aldose reductase, ROS, and c-Myc.

Conclusion: These findings support a critical role for hyperglycemic repression of miR-24 in VWF-induced pathology. miR-24 represents a novel therapeutic target to prevent adverse thrombotic events in diabetic patients.

Disclosure of Interest: None declared.

PO674-MON

The first case of an Intron retention resulting from a silent mutation in the VWF which exerts a structural impact on the 5' splice site

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Background: We identified a novel translationally silent variant (c.7464C>T, p.Gly2488) in exon 44 of von Willebrand factor gene (*VWF*) in a family with type 1 von Willebrand disease.

Aims: This study aimed to assess pathogenicity of the variant c.7464C>T with respect to RNA splicing. Furthermore, RNA secondary/tertiary structures and putative interactions with splicing regulatory proteins were analyzed *in silico* to determine the mechanisms by which this synonymous variant impairs splicing.

Methods: NA was extracted from whole blood and platelets. Complementary DNA was synthesized from RNA, and *VWF* coding region was amplified by RT-PCR. The RT-PCR products were separated on an agarose gel and sequenced to identify the variations in mRNA transcript. Human Splicing Finder prediction tool was utilized to evaluate the impact of the variant on splicing regulatory motifs. Secondary structure analysis corresponding to wild-type and mutant exons was performed on Mfold web server. Tertiary structure models were generated on an *ab initio* web server.

Results: NA transcript analysis revealed an aberrantly spliced transcript with intron 44 retained in the mRNA. Both predicted RNA secondary structures and RNA tertiary models demonstrated that this substitution in spite of its distal position from the 5' splice site (5'ss) (85 bp downstream) induces cis-alterations in pre-mRNA structure which result in the formation of a stable hairpin at the 5'ss. This hairpin sequesters the 5'ss residues involved in U1-snRNA interactions required for the first step of splicing, and thereby inhibits excision of the pre-mRNA intronic region. The aberrant transcript would result in a truncated protein due to a premature termination code in intron 44.

Conclusion: This study is the first to show the allosteric-like/far-reaching effect of an exonic variation on pre-mRNA splicing that is mediated by structural changes in the pre-mRNA.

Disclosure of Interest: None declared.

PO675-MON

Assessment of von Willebrand factor panel and platelet function in patients with moderate to severe renal insufficiency undergoing renal biopsy

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Background: Alteration of von Willebrand factor and platelet function is well-known in patients with severe renal impairment. However, the profiles of von Willebrand factor and other hemostatic markers in non-dialysis patients with moderate to severe renal insufficiency is unknown.

Aims: To investigate the characteristics of hemostatic parameters in patients with moderate to severe renal insufficiency undergoing renal biopsy.

Methods: Patients with serum creatinine (Cr) of greater than 1.5 to less than 4.0 mg dL⁻¹ scheduled for renal biopsy were enrolled. Hemostatic tests including, complete blood count (CBC), prothrombin time (PT), activated partial thromboplastin time (aPTT), vWF:Ag, vWF:RCo, vWF:CBA, FVIII:C and light-transmission platelet aggregome-

try were performed. The study was approved by the institutional ethic committee.

Results: Sixty patients were included in the preliminary analysis. The median age was 45.5 years (range 18–73). Thirty-three patients were female (55%). The mean serum Cr and glomerular filtration rate was 2.4 ± 0.6 mg dL⁻¹ and 30.3 ± 9.5 mL min⁻¹, respectively. The mean hemoglobin (Hb), hematocrit (Hct), platelet count, PT and aPTT at baseline were 11.2 ± 1.9 g dL⁻¹, $33.9 \pm 5.6\%$, $259,000 \pm 82,600$ μ L⁻¹, 11.5 ± 0.9 s and 25.7 ± 3.7 s, respectively. Increased levels of vWF:Ag, vWF:CBA and FVIII:C were observed. The mean vWF:Ag, vWF:RCO, vWF:CBA and FVIII:C were 188.9 ± 105 IU dL⁻¹, 108.5 ± 27.2 IU dL⁻¹, 137.3 ± 59.1 IU dL⁻¹ and 347.3 ± 150 IU dL⁻¹, respectively. Of fifty-six patients tested for platelet function, 4 patients (7.1%) had impaired platelet aggregation with ristocetin. A decrease in Hct of greater than 3% post renal biopsy was noted in 13 patients (21.7%). Only 2 patients required blood transfusion, including 1 patient with gross hematuria.

Conclusion: Elevation of vWF:Ag, vWF:CBA and FVIII:C as well as impaired platelet function was found in patients with moderate to severe renal insufficiency undergoing renal biopsy. Major bleeding complication is uncommon.

Disclosure of Interest: None declared.

PO676-MON

Missense mutations located in the von Willebrand factor (VWF) D1 domain cause quantitative VWF deficiency through impairment of VWF packaging into storage organelles

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Background: von Willebrand disease (VWD) results from qualitative and quantitative deficiency of von Willebrand factor (VWF). Missense mutations located in the D1 domain of VWF cause both type 1 (VWD1; mild-moderate quantitative deficiency) and type 3 (VWD3; severe deficiency) VWD.

Aims: To investigate the cellular expression of D1 domain VWD1 and VWD3 missense mutations and determine the disease mechanism(s) involved.

Methods: Bacterial pcDNA3.1 plasmids expressing either wild-type VWF cDNA (WT) or generated D1 missense mutations (p.Arg34Gly, p.Asp47His, p.Ser49Arg, p.Leu60Pro, p.Arg81Gly, p.Leu129Met) were transfected or co-transfected into HEK293T cells and the quantity of mutant VWF expressed relative to WT only was assessed using ELISA. Multimer analysis of secreted VWF was performed via electrophoresis on 1.6% (w/v) SDS-agarose gels. High-resolution microscopy of transfected HEK293 cells was performed on a Nikon TiE inverted microscope with an Andor Zyla sCMOS camera.

Results: D1 missense mutations caused a significant reduction in VWF secretion compared to WT in both the homozygous (Hom) and heterozygous (Het) state, which was more pronounced for known VWD3 mutants (~75% reduction VWD3; ~50% reduction VWD1). Expression of varying WT:mutant ratios (25:75, 75:25) confirmed that VWD3 mutants p.Arg34Gly and p.Arg81Gly had a dominant-negative effect. Mutants p.Leu129Met (reported in VWD1 and VWD3) and p.Leu60Pro caused a severe reduction (>90%) when expressed alone (Hom), but a milder ~50% reduction when co-expressed with WT (Het). Microscopy highlighted impaired Weibel-Palade body (WPB) formation in mutant-expressing cells corresponding with increased colocalisation of mutant VWF in the Golgi.

Conclusion: D1 missense mutations cause reduced VWF secretion that is more pronounced for VWD3 mutants that have a dominant-negative effect. This reduced secretion appears to result from impaired packaging of mutant VWF from the Golgi into the WPB storage organelles.

Disclosure of Interest: None declared.

PO677-MON

Minimum levels of von Willebrand factor and platelets to create occlusive high shear thrombosis

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Background: Occlusive thrombus formation on collagen can be stimulated with high shear rates. The relative role of platelet count, plasma vWF levels, and other plasma constituents can be compared by selective dilutions.

Aims: To determine the minimum concentrations of vWF and platelets for occlusive thrombosis at shear rates >3500 s⁻¹.

Methods: Occlusive thrombosis in a stenosis was produced in a microfluidic test system over collagen with initial shear rate of 3500–6000 s⁻¹. Human whole blood was diluted with normal saline by 90% or 99% and hematocrit restored by adding washed red blood cells (90%WB or 99%WB, respectively). Platelets and/or vWF (Humate-P) were added to normal levels, selectively. Microscopy images were acquired of thrombus formation over time to occlusion time (OT).

Results: Selective dilutions decreased high shear thrombus formation compared to control ($P < 0.001$). Normal human blood with no dilution occluded in 6.1 ± 2.2 min. 90%WB, reconstituted with normal levels of platelets and vWF, all formed thrombosis to occlusion (5/5, OT=13.7 $\pm$ 2.5 min). There was no occlusion in the 90%WB with no added vWF and platelets samples (0/7). 99%WB with fully reconstituted vWF and platelets showed large thrombus formation with embolization, but no occlusions (0/5). 90%WB reconstituted with only vWF occluded in 6/7 subjects (OT=16.6 $\pm$ 1.4 min), while 90%WB with only platelets occluded in 2/5 subjects (OT=27.2 $\pm$ 1.8 min). Time to occlusion was longer in 90%WB with only platelets relative to 90%WB reconstituted with only vWF ($P = 0.054$).

Conclusion: Both vWF and platelets are essential to high shear thrombosis. Normal levels of vWF are most contributory, compared to platelets and other plasma proteins. Surprisingly, fully reconstituted vWF and platelets with 99% dilutions of plasma prevented occlusion indicating the need for a third plasma factor. Efforts to control high shear thrombosis may focus on vWF levels and a third plasma factor required for occlusion instead of platelet function.

Disclosure of Interest: None declared.

PO678-MON

Aging and ABO blood type regulate von Willebrand factor and factor VIII levels through distinct patterns of both secretion and clearance

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Background: Aging and ABO blood type are major determinants of von Willebrand factor (VWF) and Factor VIII (FVIII) levels, but the mechanism(s) responsible for this influence has not been resolved

Aims: To study how aging and ABO influence VWF/FVIII levels and determine the mechanism of this effect

Methods: VWF antigen (VWF:Ag), VWF activity (VWF:RCO), VWF propeptide (VWFpp, secretion), VWFpp/VWF:Ag ratio (clearance) and FVIII (FVIII:C) levels were measured in 207 normal individuals, in 3 age groups: 52 young (7 ± 5 yrs), 42 middle-age (41 ± 6 yrs) and 113 old (71 ± 7 yrs), similar in gender and ABO blood type. Blood type A antigen on VWF (VWFa) was also measured. *P* values are shown as *(<0.05), **(<0.01), ***(<0.001).

Results: VWF:Ag increased 1.5-fold*** with age, with a clear distinction between blood type non-O (A/B) and O subjects (1.8 vs 1.2-fold, ***). The ABO effect on VWF:Ag was not observed in young (0.98-fold), but with advancing age, as the difference increased to 1.3** and 1.5-fold*** in middle and old-age individuals. Similar results were found with VWF:RCO and FVIII:C. VWFpp increased in the elderly (1.17-fold**), but only in A/B subjects (1.23 vs 1.08-fold,***). Also, VWF clearance was reduced with age in A/B individuals (0.64 vs 0.87-fold,***), suggesting roles for both secretion and clearance. VWF in the young was correlated with VWF clearance ($r = -0.87$ ***), but not with VWFpp ($r = -0.04$), independent of blood type. With aging, both VWFpp ($r = 0.82$ ***) and clearance ($r = -0.62$ ***) were associated with VWF:Ag. Moreover, VWFa correlated with VWF:Ag, VWFpp and clearance ($r = 0.25$, $r = 0.22$, $r = -0.22$ *) suggesting a novel role of ABO in VWF secretion. In the young, FVIII was not associated with VWF clearance ($r = 0.01$), but with VWF secretion ($r = 0.57$ ***); with aging, it correlated with both VWFpp and clearance ($r = 0.73$, $r = -0.40$,***).

Conclusion: This study shows that aging and ABO are two interrelated influences on VWF and FVIII levels that mediate distinct patterns of regulation through both secretion and clearance mechanisms.

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Animal Models II

PO001-TUE

Polyphosphate as a novel therapeutic for age-related macular degeneration

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Background: Age-Related Macular Degeneration (AMD) is a major cause of vision loss. Treatments are limited to ~10% of patients with the 'wet' form associated with choroidal neovascularization (CNV) and secondary bleeding. However, not all patients respond, and there are potential side effects. The pathogenesis of AMD is complex. Evidence suggests that excess complement activation contributes to the disease, damaging retinal pigment epithelial cells (RPE) and choroidal endothelial cells (CEC) of the retina. Therapies to suppress complement activation are thus being explored.

We have shown that the inorganic linear polymer, polyphosphate (polyP), interferes with complement activation via the terminal and classical pathways. We hypothesized that polyP will be protective in AMD.

Aims: To test the efficacy of polyP in a rodent model of AMD and to assess mechanisms of action.

Methods: The retinas of pigment rodents were exposed to laser injuries that induce CNV. PolyP or vehicle alone was injected intravitreally

immediately after laser injury. Five days later, eyes were excised and dissected into eye cups. Immunohistochemistry for endothelial cells and deposition of the terminal complement complex C5b-9 was performed on eye cups, followed by flatmount preparations of the immunostained choroid/RPE tissues. These were quantified by confocal microscopy and computer analysis. Effects of polyP on the growth of cultured RPE and CEC, deposition of C5b-9 and release of sC5b-9, were examined in response to H₂O₂ and complement activation.

Results: Intravitreal polyP reduced the size of laser-induced CNV lesions and deposition of C5b-9 in the choroid of rodents. Treatment of RPE and CEC with polyP dampened release of sC5b-9 and protected them against H₂O₂-induced cell death.

Conclusion: PolyP confers protection against inflammatory and oxidative cellular stresses via complement-dependent and -independent mechanisms. Further evaluation of polyP to treat AMD is ongoing.

Disclosure of Interest: None declared.

PO002-TUE

Anti-oxidant treatment improves cardiac dysfunction in Bmal1 deficient mice

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Background: Bmal1 (brain and muscle ARNT-like protein-1) deficient mice prematurely age due to an increased production of reactive oxygen species (ROS). In addition, their cardiac performance decreases with age. The effect of an anti-oxidant treatment on heart function in Bmal1 knockout (KO) mice has not been investigated yet.

Aims: To investigate whether treatment with the anti-oxidant 4-hydroxy TEMPOL improves the age-associated decline of heart function in Bmal1 KO mice.

Methods: Five-weeks-old Bmal1 KO and wild-type (WT) mice were exposed to a high fat and high cholesterol diet for 15 weeks. After 5 weeks of feeding, both genotypes were separated into 2 groups; one group received 5 mmol/l 4-hydroxy TEMPOL supplemented in the drinking water, while the other group received regular drinking water ($n = 4-6$). The effect of the anti-oxidant treatment on cardiac function was then analyzed.

Results: Echocardiographic analysis revealed that TEMPOL treatment in Bmal1 KO mice normalized heart function as evidenced by a decrease in 1) diastolic (4.4 ± 0.13 mm vs. 4.2 ± 0.044 mm, $P = 0.62$) and 2) systolic (3.1 ± 0.18 mm vs. 2.9 ± 0.077 mm, $P = 0.52$) LV internal diameters, and a decrease in 3) fractional shortening ($30 \pm 2.6\%$ vs. $32 \pm 1.8\%$; $P = 0.51$) and 4) ejection fraction ($65 \pm 3.8\%$ vs. $68 \pm 2.4\%$; $P = 0.52$). The anti-oxidant did not affect cardiac function in WT mice. Although TEMPOL did not influence cardiac ROS levels in Bmal1 KO mice, it significantly protected Bmal1 deficient cardiac telomeres from oxidation as evidenced by a reduction in the telomere damage score ($0.11 \pm 0.012\%$ vs. $0.16 \pm 0.015\%$; $P = 0.028$). In WT mice, the anti-oxidant only slightly affected this score ($0.13 \pm 0.012\%$ vs. $0.096 \pm 0.0095\%$; $P = 0.049$). In addition, TEMPOL-treated Bmal1 KO hearts showed a trend towards an increased telomere length (1.25-fold; $P = 0.10$).

Conclusion: Anti-oxidant treatment normalized heart function and reduced the premature ageing phenotype of Bmal1 KO mice, partially by detoxifying ROS.

Disclosure of Interest: None declared.

PO003-TUE

Novel mouse hemostasis model for real-time determination of bleeding time and hemostatic plug composition

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Background: Hemostasis is a rapid response by the body to stop bleeding at sites of vessel injury. Both platelets and fibrin are important for the formation of a hemostatic plug. Mice have been used to uncover the molecular mechanisms that regulate the activation of platelets and coagulation under physiological conditions. However, measuring hemostasis in mice is quite variable and current methods do not quantify platelet adhesion or fibrin formation at the injury site.

Aims: To develop a new method for the real-time visualization of hemostatic plug formation.

Methods: We describe a novel hemostasis model that uses intravital fluorescence microscopy to quantify platelet adhesion, fibrin formation, and time to hemostatic plug formation in real-time. Repeated vessel injuries of ~50 < 100 µm in diameter were induced using laser ablation technology in the saphenous vein of mice.

Results: Hemostasis in this model was strongly impaired in mice deficient in GPIIb3 or talin-1, important regulators of platelet adhesiveness. In contrast, the time to hemostatic plug formation was only minimally affected in mice deficient in the extrinsic (TFlow) or the intrinsic (FIX-/-) coagulation pathways, even though platelet adhesion was significantly reduced. A partial reduction of platelet adhesiveness using clopidogrel led to instability within the hemostatic plug, especially when combined with impaired coagulation in TFlow mice.

Conclusion: In summary, we present a novel, highly sensitive method to quantify hemostatic plug formation in mice. Based on its sensitivity towards platelet adhesion defects and its real-time imaging capability, we propose this model as an ideal tool to study the efficacy and safety of antiplatelet agents.

Disclosure of Interest: None declared.

PO004-TUE

Targeting extracellular cyclophilin a-mediated neurovascular dysfunction

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Background: The neurovascular unit (NVU) is composed of endothelium, pericytes, vascular smooth muscle cells, glia and neurons. The NVU modulates blood-brain barrier (BBB) integrity as well as cerebral blood flow (CBF). Dysfunction of this system has been implicated in many CNS disorders such as Alzheimer's disease (AD), multiple sclerosis and stroke. Cyclophilin A (CypA) is an abundant foldase that also plays a role in the initiation of inflammatory processes. Increases in the extracellular pool of CypA (eCypA) have been reported in vasculopathies such as hypertension, myocardial infarction and diabetes and eCypA has been shown to play a role in arterial thrombosis as an activator of platelets. CypA is present in cerebrospinal fluid of APOE4 carriers and preclinical data in APOE4 transgenic mice showed that elevated CypA damages the BBB. Thus, targeting eCypA to promote NVU and BBB function may be a therapeutic avenue for CNS disease.

Aims: Determine if eCypA inhibition improves cerebrovascular function in several mouse models of CNS disease.

Methods: Three models are being utilized to assess the protective effect of eCypA inhibition (eCypAi) in neurovascular disease: 1) Lipopoly-

saccharide (LPS)-induced BBB breakdown 2) Functional hyperemia in an AD mouse model and 3) ferric chloride-induced thrombosis in CNS vasculature.

Results: In the LPS model, eCypAi improved BBB integrity as assessed by leakage of fibrinogen into the brain parenchyma. Preliminary evidence suggests that two-week treatment with eCypAi restored functional hyperemia in AD transgenic mice as assessed by the whisker stimulation model of neurovascular coupling. We are currently assessing the efficacy of eCypA inhibition in prevention of cerebral thrombosis.

Conclusion: In summary, accumulating evidence suggests that inhibition of eCypA may be a novel strategy to improve vascular dysfunction associated with CNS disorders.

Disclosure of Interest: G. Suidan Employee of: Pfizer, M. Ilardi Employee of: Pfizer, A. Cameron Employee of: Pfizer, N. Kablaoui Employee of: Pfizer, K. Fonseca Employee of: Pfizer, R. Bell Employee of: Pfizer

PO005-TUE

Modification of the BDNF gene that induces depression-like states is associated to pro-inflammatory and pro-thrombotic phenotype in mice

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Background: Epidemiological studies suggest a link between depression and cardiovascular diseases (CVDs). Despite, the mechanistic correlation between CVD and depression remain poorly understood. The lack of an animal model that reproduces the pathophysiological features of depressive disorders and CVD makes this issue difficult to study.

Aims: We focused on the potential role of BDNF Val66Met polymorphism to predispose to depression disorders and to thrombosis in mice.

Methods: All procedures were performed in BDNF knock-in mice generated with a homozygous BDNF variant (Met/Met) and compared to littermate control (WT) mice. Tail suspension test and FeCl₃ arterial injury were used as model to assess depression and thrombosis respectively. The expression of different genes was analyzed by flow cytometry, Western Blot, RT-PCR and ELISA kit.

Results: BDNF^{Met/Met} mice displayed a depressive-like phenotype associated with increased platelets reactivity as suggested by their enhanced aggregation, TXA₂ biosynthesis, P-selectin and GPIIb/IIIa expression. Proteomic analysis of aorta secretome of BDNF^{Met/Met} and WT mice showed differential expression of proteins involved in the coagulation and inflammatory cascade. Similarly, different expression of gelsolin and tissue factor (TF) was observed in plasma, aortic tissue, circulating leukocytes and/or platelets. In addition, greater plasma levels of alpha-1 antitrypsin and interleukin-6, a higher erythrocyte sedimentation rate and an increased number of circulating leukocytes were detected in BDNF^{Met/Met} mice.

Conclusion: Platelet activation, alterations in coagulation pathways and changes in vessel wall morphology/composition here described in BDNF^{Met/Met} mice well recapitulate the features occurring in patients with depression or CVD. BDNFVal66Met mice might represent a good tool to understand the molecular mechanisms underlying depression and CVD.

Disclosure of Interest: None declared.

PO006-TUE

Familial feline hypertrophic cardiomyopathy: a model for evaluating platelet activation and clopidogrel therapy

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Background: To date, little is known regarding platelet activation and the benefit of anti-platelet therapy in patients with early stages of familial HCM, despite a recognized outcome of thromboembolism. A feline model of HCM is described secondary to a causative mutation (A31P) in the MYBPC3 gene. Since aortic thromboembolism is a recognized complication in HCM affected cats, this model provides a valuable scaffold for understanding the pathophysiological processes in familial HCM.

Aims: The primary aim of this study is to characterize platelet activation and aggregation in cats with the A31P mutation prior to the onset of clinical signs. The secondary aim of this study is to evaluate the efficacy of clopidogrel on platelet activation and aggregation in these cats.

Methods: Eight healthy homozygous cats with the A31P mutation and 6 wide-type cats were selected from a colony of Maine Coon-cross cats. All cats received clopidogrel (18.75 mg day⁻¹ cat⁻¹) for 14 days. Platelet activation and aggregation were assessed using flow cytometry and aggregometry before and after clopidogrel therapy.

Results: ADP-induced activation resulted in significantly elevated P-selectin expression and higher surface density of P-selectin in homozygous cats. However, no differences in ADP induced platelet aggregation were noted in untreated homozygous and wide-type cats. Clopidogrel therapy significantly suppressed ADP-induced platelet aggregation and activation in all cats.

Conclusion: Individuals with the causative A31P mutation of the MYBPC3 gene may have hyper-reactive platelets based on this animal model. Early clopidogrel therapy sufficiently suppressed platelet activation and aggregation regardless of genotype.

Disclosure of Interest: None declared.

PO007-TUE

Platelet effects of episodic ozone and pm exposure during postnatal development

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Background: Epidemiologic studies support a link between air pollutant exposures during early life and development of lung function decrements; however the underlying biological mechanisms are not fully understood. Our objective is to investigate the early life impact of episodic ozone and particulate matter exposure on hemostatic parameters.

Aims: To determine whether exposure to high levels of ozone and particulate matter during early postnatal development resulted in long-term hemostatic abnormalities.

Methods: Rhesus monkeys, born in 2008 and 2009, continuously housed outdoors were studied. 2008 animals were exposed to high particulate matter (PM) and ozone at 3 months of age, while 2009 animals were exposed to much lower PM levels. Anticoagulated peripheral blood obtained from sedated animals was used for platelet flow cytometry studies.

Results: Resting and ADP stimulated platelets from both male and female animals experiencing wildfire exposure, had significantly greater numbers of CD63 percent positive platelets, as well as increased CD63 on their surface, than their gender matched 2009

cohort. Thrombin stimulation did not result in any significant differences among the populations. Cleavage of CD42b was used as an indicator of platelet activation. All populations of animals expressed CD42b on their surface, however there were decreases in numbers of CD42b molecules on the platelet surface in both the '08F and '08M when compared with their gender matched cohort. ADP stimulation produced similar results to the resting populations. There was no difference in thrombin stimulated platelets between the 2 cohorts.

Conclusion: Our data suggest that exposure to wildfire smoke in neonatal rhesus monkey males and females results in significantly increased platelet activation which is evidence in populations of both unstimulated (resting) and ADP activated samples.

Disclosure of Interest: None declared.

PO008-TUE

Evaluation of factor xia pharmacokinetics in mice with a thrombin generation assay

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Background: Trace amounts of activated Factor XI (FXIa) were found in plasma of patients with various thrombotic conditions, suggesting that FXIa is either a by-product of or a participant in the thrombosis development. FXIa contamination has also been found in several plasma-derived immune globulin products associated with increased rates of thrombotic adverse events. Risk of FXIa-induced thrombosis may depend on FXIa circulatory half-life but FXIa pharmacokinetics has never been studied.

Aims: Develop a micro-volume assay for evaluation of FXIa pharmacokinetics in mice.

Methods: Thrombin generation assay was optimized for evaluation of micro-samples (20 µL) of mouse plasma. Each sample was tested twice, with and without anti-FXI inhibitory antibody AHXI-5061. Blood samples were collected from mice after intravenous administration of the highest non-toxic dose of human FXIa, determined in preliminary dose-escalation studies.

Results: Specificity of anti-FXI antibody-based thrombin generation assay was confirmed *in vitro* as well as by samples collected after mock administrations in mice. FXIa-dependent thrombin generation activity was detected as early as 10 min after administration, and declined steadily over 1, 2, and 8 h time points, reaching the baseline thrombin generation levels after 24 h.

Conclusion: Circulating FXIa activity can be detected many hours after the administration, confirming that FXIa can be a marker of both ongoing and past thrombotic events. Analysis of FXIa pharmacokinetics may be helpful in the evaluation of relative thrombogenic potentials of FXIa-containing blood-derived products, e.g., intravenous, subcutaneous and intramuscular immune globulins. Further studies are needed to clarify the relationship between plasma level of FXIa and the risk of thrombotic event development.

Disclaimer: This is an informal communication and it represents authors' best judgment. These comments do not bind or obligate FDA.

Disclosure of Interest: Y. Liang: None Declared, E. Struble: None Declared, W. Chang: None Declared, D. Scott: None Declared, M. Ovanesov Grant/Research Support from: FDA Office of Women's Health

Antiplatelets agents II

PO009-TUE

Resistance to anti-platelet agents in patients with ischemic stroke

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Background: Aspirin and Clopidogrel are currently the most prevalent antiplatelet agents [APA] used in the prevention of ischemic, non-cardioembolic strokes. However, variable response to antiplatelet therapy needs monitoring by platelet function studies.

Aims: To explore the prevalence of resistance to anti-platelet therapy with aspirin and/or clopidogrel among patients with ischemic stroke and correlate its relation to stroke recurrence.

Methods: Adult patients with ischemic stroke treated either with Aspirin or Clopidogrel or both were enrolled. Platelet function was studied by light transmission aggregometry [LTA] using ADP and arachidonic acid as the agonist and PFA-100 screen. Aspirin resistance was defined as a mean platelet aggregation $\geq 20\%$ with 0.5 mg mL^{-1} arachidonic acid and/or $\geq 70\%$ with $5 \text{ } \mu\text{mol L}^{-1}$ adenosine diphosphate by LTA. Clopidogrel resistance was defined as a mean platelet aggregation $\geq 40\%$ with $5 \text{ } \mu\text{mol L}^{-1}$ adenosine diphosphate by LTA. Frequency of resistance to APA among patients with and without stroke recurrence over the period of antiplatelet therapy was compared.

Results: Of the 23 patients studied [Mean Age: $67 \pm 10 \text{ yr}$. M: F::11:12], 21 were on aspirin and 11 on clopidogrel. Of these, 9 were on dual antiplatelet therapy. Overall, 3 patients on aspirin and 4 patients on clopidogrel had resistance to anti-platelet effect (30%). While the failure of aspirin effect was observed in 3 patients with monotherapy, 3/4 of the failures with clopidogrel occurred in patients on dual APA therapy. Amongst the 14 patients (61%) who had recurrent stroke, 5 (36%) had APA resistance as compared to 2 (22%) patients without recurrent stroke [$P = 0.95$, Chi Square test].

Conclusion: Resistance to the anti-platelet effect of clopidogrel (36%) appears to be more frequent than that due to aspirin (14%) in Omani population. Resistance to APA is more prevalent in patients with recurrent stroke [1:1.6] but larger studies are necessary to explore the correlation of resistance to APA with recurrence of cardiovascular events.

Disclosure of Interest: None declared.

PO010-TUE

Mechanism of antiplatelet action induced by guanosine via increase in camp levels and its antithrombotic activity

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Background: Guanosine is a natural bioactive compound and endogenous nucleoside that has been identified in fruits and vegetables (tomatoes, potatoes and apples, among others). Guanosine exhibits antiplatelet activity. However, the specific mechanisms by which this inhibition occurs is not completely established.

Aims: The main aim of this work was to investigate antiplatelet action mechanisms and antithrombotic activity of guanosine.

Methods: Guanosine ($10 \text{ a } 500 \text{ } \mu\text{mol L}^{-1}$) was evaluated on i) Platelet P-selectin expression and GPIIb/IIIa activation by flow cytometry, ii) Platelet ATP secretion and aggregation induced by ADP, collagen and arachidonic acid, iii) Platelet adhesion and aggregation under controlled flow conditions, iv) Intraplatelet cAMP levels and release of

sCD40L, IL-1 β , TGF- β 1 and CCL5, v) Phosphodiesterase 3A (PDE3A) activity and phosphorylation of the cAMP-dependent Protein Kinase (PKA), and vi) Antithrombotic activity (200 mg kg^{-1}) in a murine model. The guanosine docking with respect to adenosine A2A receptor was determined by homology.

The protocols were authorized by the ethics committee of the Universidad de Talca in accordance with the Declaration of Helsinki.

Results: Guanosine concentration-dependently ($10 \text{ a } 500 \text{ } \mu\text{mol L}^{-1}$) inhibited P-selectin expression and GPIIb/IIIa activation, platelet ATP secretion and aggregation induced by ADP, collagen and arachidonic acid, and diminished platelet adhesion and aggregation under controlled flow conditions on collagen. At these concentrations guanosine significantly decreased the release of inflammatory mediators from platelet activation (sCD40L, IL-1 β , TGF- β 1 and CCL5). Furthermore, guanosine increased intraplatelet cAMP levels/PKA activation and is compatible to the active site of the adenosine A2A receptor as revealed through molecular modeling. Moreover, guanosine had a potent antithrombotic activity.

Conclusion: Antiplatelet and antithrombotic effects of guanosine are associated with the adenosine A2A receptor and cAMP/PKA signaling pathway.

Disclosure of Interest: None declared.

PO011-TUE

Impact of non-inhibited platelet supplementation on platelet reactivity in patients treated with prasugrel or ticagrelor for an acute coronary syndrome: an ex-vivo study

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Background: Managing bleeding in patients receiving P2Y₁₂ inhibitors is challenging. Few data are available regarding the efficacy of platelet transfusion in patients treated with prasugrel or ticagrelor.

Aims: The aim of this study was to evaluate the minimal amount of platelet supplementation necessary to restore platelet reactivity in platelet-rich plasma (PRP) of patients treated with aspirin and a prasugrel or ticagrelor loading dose for an acute coronary syndrome.

Methods: PRP samples from patients were mixed *ex-vivo* with increasing proportions of pooled PRP from healthy volunteers. Platelet reactivity was challenged with adenosine diphosphate (ADP), arachidonic acid, collagen or thrombin receptor activating peptide using light transmission aggregometry. The primary endpoint was the proportion of patient samples recovering an ADP-induced maximal aggregation (ADP-Aggmax) value above 40%.

Results: In patients treated with prasugrel ($n = 32$), ADP-Aggmax increased progressively with supplements of pooled PRP, with an average increase of 7.9% (95% CI [7.1; 8.8], $P < 0.001$) per 20% addition of non-inhibited platelets. The addition of 60% non-inhibited platelets was associated with 90% of patients reaching the primary endpoint. In patients treated with ticagrelor ($n = 15$), ADP-Aggmax did not significantly increase with any level of supplements.

Conclusion: *Ex-vivo* addition of non-inhibited platelets significantly improved ADP-Aggmax in patients treated with prasugrel with a dose dependent effect. There was no evidence of such a reversal in patients treated with ticagrelor. These results suggest that platelet transfusion may be more effective in blunting bleeding in patients treated with prasugrel, than those treated with ticagrelor.

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PO012-TUE

The effects of two commonly used herbal products on haemostatic system and their interactions with aspirin

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Background: The use of herbal products as alternative medicine is prevalent worldwide. *Curcuma longa* (CL) and *Angelica sinensis* (AS) are two commonly used herbs with purported 'blood activating' properties. Their effects on haemostasis and interaction with aspirin are unknown.

Aims: To investigate the effects of CL and AS on platelet function, thrombin generation and interaction with aspirin.

Methods: Randomized, double-blind, placebo-controlled crossover study was carried out with 3 phases of 3-week intervention comprising of herbal capsule only (CL 500 mg daily or AS 1000 mg daily), aspirin only (100 mg daily) and aspirin + herbal capsule, with 2 weeks' wash-out period in between. Blood samples were analysed at baseline and after each phase for platelet function (PFT) using light transmission aggregation and thrombin generation (TG) on platelet poor plasma. Clinical events were recorded.

Results: 25 healthy volunteers were recruited for each of the CL and AS groups. CL and AS only affected the arachidonic acid (AA)-induced aggregation. The median AA-induced percentage (%) aggregation and AUC (5th-95th percentile) in the CL group were 92.5% (9.8% – 125%) ($P < 0.05$) and 358.3 (53.6 – 695.1) respectively, compared to baseline [100.0% (79.0%–123.2%); AUC=402.6 (159.7–639.7)]. The median AA-induced %aggregation and AUC in the AS group were 91% (30% – 105.9%) and 400.8 (119.5 – 516.4) ($P < 0.05$) respectively, compared to baseline [93.5% (70.8%–107.0%); AUC=432.3 (339.3–533.7)]. The herbs did not affect TG parameters significantly from the baseline. The addition of herbs did not further aggravate aspirin-induced platelet dysfunction or TG parameters. No clinical events were reported for all groups.

Conclusion: CL and AS suppressed AA-induced PFT without causing any significant adverse clinical event. The widely held beliefs of 'blood-activating' properties of CL and AS were not demonstrated through PFT and TG. The concurrent usage of CL or AS with aspirin appears to be safe.

Disclosure of Interest: None declared.

PO013-TUE

Efficacy of platelet inhibition with prasugrel in patients with acute myocardial infarction undergoing therapeutic hypothermia after cardiopulmonary resuscitation

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Background: Acute myocardial infarction (AMI) is the leading cause for out-of-hospital cardiac arrest. Therapeutic hypothermia improves neurological outcome in combination with early revascularization, but reduces clopidogrel responsiveness. The more potent thienopyridine prasugrel has not yet been sufficiently evaluated during therapeutic hypothermia.

Aims: To assess the responsiveness to prasugrel in patients undergoing therapeutic hypothermia.

Methods: We investigated 23 consecutive AMI patients (61 ± 11 years) following out-of-hospital resuscitation undergoing revasculari-

zation and therapeutic hypothermia. Prasugrel efficacy was assessed by the platelet-reactivity-index (PRI) before and 2, 4, 6, 12, 24, 48, and 72 h following a loading dose of 60 mg via a gastric tube.

Results: Prasugrel significantly reduced PRI despite hypothermia ($P = 0.0002$). Mean PRI ($\pm$ SD) was $70 \pm 12\%$ before and $60 \pm 16\%$ (2 h, ns), $52 \pm 21\%$ (4 h, $P < 0.05$), $42 \pm 26\%$ (6 h, $P < 0.01$), $37 \pm 21\%$ (12 h, $P < 0.01$), $27 \pm 23\%$ (24 h, $P < 0.01$), $18 \pm 14\%$ (48 h, $P < 0.01$), and $13 \pm 10\%$ (72 h, $P < 0.01$) after loading. Sufficient platelet inhibition occurred later compared to stable AMI patients, however, high on-treatment platelet reactivity significantly decreased over time and was nonexistent after 72 h (PRI>50%: 2 h: 72%, 4 h: 52%, 6 h: 43%, 12 h: 29%, 24 h: 17%, 48 h: 5%, 72 h: 0%). There was no relation between 30d mortality rate (31%) and PRI values.

Conclusion: Prasugrel significantly reduced platelet reactivity even during vasopressor use, analgosedation and therapeutic hypothermia. Despite a significant delay compared to stable AMI patients, sufficient platelet inhibition was reached in 83% of patients within 24 h. Therefore, prasugrel administration via gastric tube might be a useful therapeutic strategy in these patients at high risk, providing potent and effective P2Y₁₂ inhibition.

Disclosure of Interest: A. Schäfer Speaker Bureau of: lecture fees for Daiichi-Sankyo/Eli Lilly, U. Flierl: None Declared, J. Bauersachs: None Declared.

PO014-TUE

Antibody targeted, thrombin-sensitive polymer capsules for urokinase delivery

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Background: Plasminogen activators such as Urokinase generate plasmin, which lyses fibrin and restores perfusion. However, thrombolytic drugs can lead to systemic plasminogen activation and plasmin generation causing severe and potentially life threatening bleeding complications leaving many patients untreated.

Aims: Nanotechnology has the potential to overcome those limitations providing safe and efficient thrombolysis. We set out to develop targeted nanoparticles that can encapsulate biomolecules such as Urokinase thereby preventing systemic plasminogen activation in the circulation. Here we present the development of an antibody targeted thrombin sensitive capsule system loaded with Urokinase.

Methods: Biocompatible polymers were layered on Urokinase filled silica particles and crosslinked with a thrombin sensitive peptide linker. To provide thrombus targeting, an activation specific single-chain antibody against platelets was equipped with an orthogonal chemistry group using enzymatic bioconjugation and attached to the capsules surface using site-specific chemistry.

Results: After removal of the sacrificial template, loading of active Urokinase was confirmed using dye labelled enzyme as well as chromogenic Urokinase assays. Rapid, dose dependent capsule degradation was observed with L-amino acid linked particles but not when a D-amino acid linker was used. This was independent of the surface attachment with the antibody. Platelet targeting was confirmed by flow cytometry and flow chamber assays.

Conclusion: We have developed a thrombin sensitive nanocapsule system for targeted delivery of thrombolytic drugs. Protecting drugs in the circulation and only releasing active biomolecules at the site of action could provide significant progress in moving the field out of its

current stagnation. The system presented here could provide safe and potent thrombolysis for myocardial infarction, stroke and other acute thrombotic events.

Disclosure of Interest: None declared.

PO015-TUE

Platelet transfusion might be a potent antidote for thienopyridines, but not for ticagrelor

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Background: Inhibition of platelet P2Y₁₂ ADP receptors in patients with acute coronary syndrome or after percutaneous coronary intervention effectively reduces the rate and extent of subsequent cardiovascular events. The reversal of antiplatelet drug effects is an important issue at trauma or emergency departments as a higher bleeding tendency is correlated with higher morbidity and mortality. Platelet transfusions are frequently administered to counteract platelet defects.

Aims: In our *ex vivo* study we mimicked platelet transfusion by adding fresh (donor) platelets to platelets from cardiovascular patients, who were on stable medication with prasugrel, clopidogrel or ticagrelor. The ongoing study is intended to verify, whether the reversible or irreversible mode of action of the drug has an influence on the ability of fresh platelets to restore platelet function in such mixtures.

Methods: Blood was taken from patients 4 h after administration of a P2Y₁₂ antagonist in order to mimic a situation that might require platelet transfusion. We have studied so far 34 patient/donor pairs that differed in their HLA-A2/A28 status allowing a differentiation of platelets in a mixture by using an A2/A28-specific fluorochrome-coupled antibody with flow cytometric analysis. Under these conditions we were able to measure ADP-induced fibrinogen binding and α -granule release for the whole platelet population as well as for the individual platelet populations from donors or patients in one sample.

Results: We found that the reactivity of donor platelets to ADP was not affected when added to platelet-rich plasma from prasugrel- ($n = 12$) or clopidogrel-treated ($n = 8$) patients. In contrast, the reactivity of the donor platelets was dramatically reduced when added to platelet-rich plasma from all patients ($n = 14$) receiving ticagrelor ($P < 0.001$).

Conclusion: These data suggest that in contrast to clopidogrel and prasugrel, the activity of ticagrelor may not be directly and/or immediately reversed by platelet transfusion.

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PO016-TUE

Ticagrelor reversal: *in vitro* assessment of various hemostatic agents

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Background: Ticagrelor, a reversible P2Y₁₂ inhibitor, is recommended for the treatment of acute coronary syndrome. Management of bleeding complications is challenging as no antidote is available. Platelet transfusion, usually proposed as a reversal strategy for antiplatelet drugs, is poorly effective since circulating ticagrelor and its active metabolite inhibit transfused platelets (1). Thus, effective strategy is critically needed to manage ticagrelor-induced bleeding.

Aims: To investigate *in vitro* the efficacy of 4 hemostatic drugs (HD), namely recombinant activated factor VII (rFVIIa), fibrinogen (Fib), tranexamic acid (TXA) and factor XIII (FXIII) used at clinically-used concentrations, to restore hemostatic capacities inhibited by ticagrelor.

Methods: Blood from healthy volunteers was spiked with ticagrelor (0 or 3.25 μ M, $n > 5$) then supplemented by either HD or control. HD efficacy was investigated with 20 μ M ADP-induced platelet aggregation measured by impedance on whole blood and by light transmission on platelet rich plasma (PRP), thrombolastography Platelet Mapping assay (TEG-PM)(ADP 2 μ M) and thrombin generation assay (TGA) on PRP (ADP 20 μ M).

Results: Ticagrelor strongly inhibited ADP-induced platelet aggregation and decreased the clot strength (MA) in TEG-PM_{ADP}. None of the tested HD corrected these parameters. However, using TEG-PM_{thrombin} (kaolin/calcium), rFVIIa shortened coagulation time R ($P = 0.03$), Fib increased MA ($P = 0.03$) whereas FXIII decreased LY30 ($P = 0.03$). Furthermore, rFVIIa shortened time to peak prolonged by ticagrelor in TGA ($P = 0.03$). No effect of TXA was observed.

Conclusion: Whereas none of the tested HD corrected ticagrelor effects on ADP-induced aggregation, rFVIIa shortened coagulation time, Fib increased clot firmness and FXIII decreased fibrinolysis. Although these HD do not have direct effect on platelets they could compensate ticagrelor effects by acting on fibrinofibrinolysis.

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PO017-TUE

Two disintegrins, TFV-1 and TFV-3, purified from trimeresurus flavoviridis snake venom, exhibit distinct effects on platelet activation

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Background: Disintegrins are potent small-mass platelet inhibitors found in snake venoms, which are GPIIb/IIIa antagonist and potential antithrombotic agents. Current GPIIb/IIIa antagonists have the life-threatening adverse effect of causing thrombocytopenia and bleeding. There were two disintegrins, TFV-1 and TFV-3, purified from *Trimeresurus flavoviridis* snake venom, with different properties.

Aims: We compared if any different effects on platelet activation exist between TFV-1 and TFV-3.

Methods: Gel filtration and reverse-phase HPLC were used for purification and MALDI-TOF for molecular weight determination. Lumi-Aggregometer to examine platelet aggregation. Flow cytometric analysis for indirect binding of disintegrins and GPIIb/IIIa monoclonal antibodies to platelets.

Results: The molecular weight of TFV-1 and TFV-3 were determined as 7310 Da and 7646 Da by MALDI-TOF, respectively. Both TFV-1 and TFV-3 concentration-dependently inhibited platelet aggregation in human platelet-rich plasma and washed platelet suspension. The IC₅₀ values of TFV-1 and TFV-3 for platelet aggregation induced by ADP (20 μ M), thrombin (0.1 unit mL⁻¹) and collagen (10 μ g mL⁻¹) were estimated to be 0.73 and 0.23 μ g mL⁻¹, 0.47 and 0.23 μ g mL⁻¹, and 0.52 and 0.22 μ g mL⁻¹, respectively. In clot retraction test, TFV-1 promoted thrombin-induced clot retraction while TFV-3 inhibited it. On other hand, TFV-3 induced platelet aggregation in the presence of AP2, a GPIIb/IIIa monoclonal antibody, due to Fc γ RIIa recruitment, but TFV-1 did not. TFV-3 significantly inhibited 7E3, a mAb raised against GPIIb/IIIa, binding to GPIIb/IIIa, but TFV-1 did not.

Conclusion: Two disintegrins, TFV-1 and TFV-3 exhibit distinct binding epitopes on GPIIb/IIIa and different effects on platelet activation process. These differences between TFV-1 and TFV-3 may provide valuable information for the development of GPIIb/IIIa inhibitors and the mechanisms involved in GPIIb/IIIa inhibitor-induced thrombocytopenia and bleeding.

Disclosure of Interest: None declared.

PO018-TUE

Platelet inhibition monitoring with thromboelastography (TEG) platelet mapping in pediatric patients with ventricular assist device

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Background: Berlin Heart (BH) is the first pulsatile ventricular assist devices suitable for small children. Thrombosis in this context remains an issue. Thus, a multi-system anticoagulation protocol is recommended, including dual antiplatelet therapy with dipyridamole and aspirin (ASA). TEG Platelet Mapping (TEGPM) is required by BH's manufacturer to monitor platelet inhibition (PI).

Aims: To assess if TEGPM is reliable to monitor PI induced by dipyridamole and ASA in children on BH support.

Methods: From 2009 to 2015, we have followed 6 patients (2 to 10 years) implanted with BH. PI was assessed with TEGPM, used as recommended, and/or light transmission aggregometry (LTA) with 5 μ M ADP and 1 mM AA. According to the guidelines of BH, target PI for dipyridamole and ASA is reached with TEGPM if G_{ADP}<6 dynes cm⁻² and AA inhibition >70% respectively and with LTA if maximal aggregation (MA) with ADP is <50% and with AA <30% respectively. Results are given as median [min-max].

Results: Two of the six children did not receive antiplatelet because one died shortly after implantation and the other was soon transplanted. Three of them were successfully bridged to transplantation, and one had myocardial recovery. In one child, right ventricle was changed twice. Monitoring of children receiving dual antiplatelet therapy was done by LTA (3 patients) and/or by TEGPM (4 patients). Overall, 15 LTA and TEGPM were done in parallel. The agreement between both methods was 60% for AA (9/15) but only 29% for ADP

(4/14). In most cases of discordant results for ADP, TEGPM showed PI (G_{ADP} 3.8 dynes cm⁻² [1.8–9.4]) while LTA showed high platelet reactivity (MA 81.5% [28–100]). For two children, the clot viscoelastic maximum amplitude often showed an abnormal pattern, suggesting spontaneous platelet activation.

Conclusion: TEGPM and LTA often lead to different decisions for adjusting doses of dipyridamole. In some patients, abnormal TEGPM pattern altered the assessment of AA or ADP receptor pathway.

Disclosure of Interest: None declared.

PO019-TUE

The prognostic utility of platelet function testing for the detection of 'aspirin resistance' in patients with established cardiovascular disease

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Background: Aspirin is well established for secondary prevention of cardiovascular disease. However, a proportion of patients suffer repeat cardiovascular events despite being prescribed aspirin treatment. It is uncertain whether this is due to an inherent inability of aspirin to sufficiently modify platelet activity.

Aims: To investigate whether insufficient platelet function inhibition by aspirin ('aspirin resistance'), as defined using platelet function tests (PFTs), is linked to the occurrence of adverse clinical outcomes.

Methods: Standard systematic review methods were used for identifying clinical studies where aspirin was used as the sole antiplatelet agent in secondary prevention of cardiovascular disease ($n = 58$). Unadjusted and adjusted odds and hazard ratios for the association between aspirin resistance for different PFTs and clinical outcomes were extracted or calculated (Registered as PROSPERO 2012: CRD42012002151).

Results: The main PFTs used were light transmission aggregometry (LTA), VerifyNow Aspirin, thromboxane metabolites, PFA-100, whole blood aggregometry and thromboelastography. A trend for more clinical events in groups designated 'aspirin resistant' was seen for some PFTs (LTA, VerifyNow Aspirin, PFA-100, thromboxane metabolite measurement). However, study-level effect sizes were generally small and absolute outcome risk was not substantially different between 'aspirin resistant' and 'aspirin sensitive' designations.

Conclusion: Although evidence indicates that some PFTs may have some prognostic value, methodological and clinical heterogeneity between studies and different approaches to analyses create inconsistency in prognostic results. Adequately powered primary studies are needed, using standardised methods of measurements to evaluate the prognostic ability of each test in the same population(s). For any PFT to inform individual risk prediction, it will likely need to be considered in combination with other prognostic factors, within a prognostic model.

Disclosure of Interest: None declared.

PO020-TUE

A time course study of high on treatment platelet reactivity in ACS patients on dual antiplatelet therapy

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Background: Limited data are available on the natural history of high on treatment platelet reactivity (HPR) by arachidonic acid (AA) and ADP – markers of unfavorable prognosis in ACS patients –.

Aims: We evaluated the time-course of HPR by ADP and AA during 1 year of follow-up of a cohort of ACS patients on dual antiplatelet treatment.

Methods: In 130 ACS patients, we assessed HPR by ADP (LTA by 10 μ M ADP $\geq$ 70%) and AA (LTA by 1 mmol AA $\geq$ 20%) measuring platelet function in the acute phase (T0), at 6 months (T1) and 1 year (T2).

Results: We identified *persistent* (HPR at T0, T1 and T2), *acute non persistent* (HPR only at T0), and *late* (HPR only at T1 or T2). Patients with *persistent* and *acute non persistent* HPR by ADP were more frequently females. Diabetes was more prevalent in patients with *acute non persistent* HPR by ADP. Patients carrying CYP2C19*2 variant were more prevalent in the group of *persistent* HPR (40%). Significant higher values of immature platelet fraction (IPF) at 12 months were present in patients with *late* HPR. This was the only variable significantly associated with late HPR by ADP at multivariate analysis (OR=1.28 (1.1–1.6), P = 0.049). Patients with *persistent* HPR by AA were significantly older, more frequently females and diabetic. At multivariate analysis, female sex was the only parameter associated with *late* (OR=3.8 (1.1–14.3), P = 0.045) and *acute non persistent* HPR (OR=4.2 (1.1–15.8), P = 0.035).

Conclusion: About 25% of 130 patients has *persistent* HPR; they are more frequently females, diabetics or carriers of CYP2C19*2. The occurrence of an inflammatory state, documented by the increase of IPF, is associated with the occurrence of *late* HPR.

Disclosure of Interest: None declared.

PO021-TUE

Inhibition of platelet aggregation by GLS-409, an antagonist of both P2Y₁ and P2Y₁₂, is distinct from inhibition by selective P2Y₁ and P2Y₁₂ antagonists

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Background: Dual antiplatelet therapy with aspirin (ASA) and an adenosine diphosphate (ADP) receptor inhibitor is the standard of care to prevent thrombotic events in patients with acute coronary syndromes. Currently approved ADP receptor antagonists target only the P2Y₁₂ receptor. In contrast, diadenosine tetraphosphate (Ap₄A) and its derivatives also target the second human platelet ADP receptor, P2Y₁.

Aims: To determine the effects on platelet aggregation, pre- and post-ASA ingestion, of simultaneous inhibition of both P2Y₁ and P2Y₁₂ by a new Ap₄A derivative (GLS-409) as compared to selective P2Y₁₂ inhibition with cangrelor and selective P2Y₁ inhibition with MRS2179.

Methods: Blood was drawn from 6 healthy individuals before and 2 h after the ingestion of 325 mg ASA. Platelet-rich plasma (PRP) and whole blood was incubated (37°C, 15 min) with different concentrations of GLS-409, cangrelor or MRS2179, then platelet aggregation in response to ADP, collagen and thrombin receptor-activating peptide (TRAP) was determined by 96-well optical and whole blood impedance aggregometry.

Results: GLS-409 inhibited ADP-, collagen- and TRAP-stimulated platelet aggregation in PRP and in whole blood. ASA ingestion reduced the half-maximal inhibitory concentration for GLS-409 inhibition of TRAP- but not ADP- or collagen-stimulated aggregation. Cangrelor produced similar results, except that ASA did not alter cangrelor inhibition of TRAP-stimulated aggregation. MRS2179 did not inhibit TRAP-stimulated platelet aggregation and showed increased inhibition of collagen-stimulated platelet aggregation in PRP and whole blood following ASA ingestion.

Conclusion: The pattern of inhibition of platelet aggregation by GLS-409, an antagonist of both P2Y₁ and P2Y₁₂, is distinct from that produced by selective P2Y₁ and P2Y₁₂ antagonists. These unique *ex vivo* properties suggest GLS-409's *in vivo* effects may also be distinct from currently available selective P2Y₁₂ antagonists.

Disclosure of Interest: T. Gremmel: None Declared, I. Yanachkov Employee of: GL Synthesis, G. Wright Employee of: GL Synthesis, A. Michelson Grant/Research Support from: GL Synthesis, Lilly/Daiichi Sankyo, Pfizer, Bristol-Myers Squibb, Consultant for: Lilly, A. Frelinger Grant/Research Support from: GL Synthesis, Lilly/Daiichi Sankyo, Pfizer, Bristol-Myers Squibb.

PO022-TUE

P2Y₁₂ is not involved in the initiation and stabilization of mural thrombi after laser injury of an artery

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Background: We have previously shown that tissue factor (TF)-positive neutrophils bind to endothelial cells at the site of vessel wall injury and initiate thrombus formation. After laser injury, neutrophil-localized TF activity triggers platelet activation via thrombin formation followed by fibrin formation. The irreversible P2Y₁₂ antagonist clopidogrel and the reversible P2Y₁₂ antagonist ticagrelor are both approved drugs for prevention of major adverse vascular events in patients with acute coronary syndromes.

Aims: Comparison of clopidogrel with two reversible P2Y₁₂ antagonists, ticagrelor and ACT-246475, in injury-induced and neutrophil-mediated thrombus formation.

Methods: Laser-induced thrombus formation was studied using real time intravital microscopy in mice. A novel method to quantify the stability of mural thrombi was applied.

Results: Comparison of irreversible and reversible P2Y₁₂ antagonists confirmed a dose-dependent inhibition of laser-induced platelet thrombi. Clopidogrel and ticagrelor, but not ACT-246475, decreased the number of adherent neutrophils and the extent of thrombus-associated fibrin. In addition, clopidogrel and ticagrelor, but not ACT-246475, reduced the stability of mural thrombi as quantified by increased detachment of platelet aggregates from mural thrombi.

Conclusion: The P2Y₁₂ antagonist ACT-246475 efficiently inhibited thrombus growth without affecting neutrophil adhesion, fibrin formation and stability of mural thrombi. Therefore, the three P2Y₁₂ antagonists clearly differ in their off-target effects. In addition, the data suggest that P2Y₁₂ is not involved in the initiation and stabilization of platelet thrombi formed after laser injury. Increased stability of mural

thrombi, as observed in the presence of ACT-246475, might be beneficial for an antithrombotic agent.

Disclosure of Interest: R. Darbousset Grant/Research Support from: Actelion Pharmaceuticals, E. Caroff Employee of: Actelion Pharmaceuticals, F. Hubler Employee of: Actelion Pharmaceuticals, M. Riederer Employee of: Actelion Pharmaceuticals, L. Panicot-Dubois Grant/Research Support from: Actelion Pharmaceuticals, C. Dubois Grant/Research Support from: Actelion Pharmaceuticals.

PO023-TUE

Safety of rivaroxaban: effects on platelet function

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Background: Rivaroxaban (RIV) is a direct inhibitor of FXa. It has been suggested that RIV may have antiplatelet effects. However, information about the dose-dependent antiplatelet effect of RIV is quite scarce.

Aims: To learn about the likely antiplatelet effects of 4 different doses of RIV on platelet function.

Methods: We included adult patients, men and women who received RIV due to specific indications (2.5, 10, 15, and 20 mg). All patients gave informed consent. Platelet function tests were performed before and 30 days after using RIV. Blood samples were drawn 24 h after the last dose of the drug (12 h for the 2.5 mg group). Platelet function was evaluated by means of platelet aggregometry using adenosine diphosphate (ADP;10 μ M), epinephrine (EPI;10 μ M), and arachidonic acid (AA;0.5 mM). The study was performed according to the Declaration of Helsinki and it was approved by the Ethical Committee of our Institution.

Results: Eighty samples (46 women; 34 men) were analyzed (20 group⁻¹). Mean age for men and women was 47 and 49 year-old, respectively. Mean $\pm$ SD basal vs. post-treatment platelet aggregation for all groups were: 2.5 mg: AA (76 $\pm$ 15 vs. 73 $\pm$ 17%), ADP (77 $\pm$ 18 vs. 71 $\pm$ 16%), EPI (71 $\pm$ 19 vs. 74 $\pm$ 18%); 10 mg: AA (79 $\pm$ 11 vs. 82 $\pm$ 9%), ADP (77 $\pm$ 16 vs. 70 $\pm$ 16%), EPI (79 $\pm$ 18 vs. 80 $\pm$ 19%); 15 mg: AA (79 $\pm$ 16 vs. 81 $\pm$ 19%), ADP (78 $\pm$ 18 vs. 75 $\pm$ 14%), EPI (76 $\pm$ 19 vs. 79 $\pm$ 17%); 20 mg: AA (76 $\pm$ 17 vs. 78 $\pm$ 16%), ADP (74 $\pm$ 14 vs. 75 $\pm$ 12%), EPI (70 $\pm$ 20 vs. 80 $\pm$ 19%). Differences before and after using RIV were not significant ($P \geq 0.05$). Also, there were not significant differences between post-treatment results among the 4 groups ($P \geq 0.05$).

Conclusion: Even at the highest approved doses RIV does not affect the platelet aggregation. These results add information about the safety of the drug and strongly suggest that it should not be used alone in the treatment or prevention of diseases in which platelets play an essential role.

Disclosure of Interest: None declared.

PO024-TUE

The start-antiplatelets register: a multicenter observational prospective study to assess the risk-benefits of antithrombotic therapy in acs patients

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Background: The treatment of ACS has been modified in recent years by the introduction of the more potent P2Y₁₂ inhibitors. Scarce data are available on the adherence to guideline recommendations in the real world, and on the follow-up of ACS patients. The significant prevalence of atrial fibrillation and of other conditions requiring an oral anticoagulant therapy in ACS patients, raise complex clinical decision problems for the association of antithrombotic therapies.

Aims: To register type and length of antiplatelet therapies in ACS patients; to evaluate bleeding and thrombotic risk at 6 and 12 month follow-up

Methods: Participant centers to the START-ANTIPLATELETS Register, a collaborative observational study, enroll all patients who meet predefined eligibility criteria (ACS). Data of each individual patient, including diagnosis, underlying conditions, type of antiplatelet treatment and causes of its variation, or of temporary or definitive interruption, and finally events occurring during 6- and 12-month follow-up, are collected on the web-site of the register.

Results: 175 pts were registered so far (41 F; 134 M/age: 67 $\pm$ 12.5 yrs). Diagnoses included: 75 STEMI; 74 NSTEMI and 26 UA. 141 (80.5%) pts underwent PCI; 130 were revascularized with stent implantation (127/130 DES); 29 (16.5%) were medically treated and 9 (5.1%) underwent coronary artery bypass graft surgery. At discharge antithrombotic therapy included: aspirin in 171/175 (98%); P2Y₁₂ inhibitors in 161/175 (92%): 77 (47.8%) clopidogrel; 65 (40.3%) ticagrelor; 19 (11.8%) prasugrel. 15/175 (8.6%) pts were treated with a triple antithrombotic therapy: 13 patients with VKA+aspirin+clopidogrel; 1 with dabigatran+aspirin+clopidogrel and 1 with ticagrelor+VKA+aspirin.

Conclusion: START ANTIPLATELETS Register, a multicenter observational prospective register, will allow us to enhance our understanding of the risk-benefits of the antithrombotic therapy in ACS patients.

Disclosure of Interest: R. Marcucci Speaker Bureau of: bayer; Astra Zeneca; Merck; Pfizer; Eli Lilly; Daichy Sankyo, V. pengo: None Declared, G. Patti: None Declared, P. Gresele: None Declared, E. Antonucci: None Declared, S. Valente: None Declared, A. Bracco: None Declared, T. Fierro: None Declared, I. Cavallari: None Declared, G. F. Gensini: None Declared, G. Palareti: None Declared

Arterial Vascular disorders – clinical II

PO025-TUE

Association of the mean platelet volume (MPV) with the diagnosis of acute coronary syndromes (ACS) among patients who present with chest pain at the emergency room (ER)

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Background: Mean platelet volume (MPV), an index for platelet size, is associated with acute coronary syndromes (ACS).

Aims: The main objective of this study is to determine whether there is an association between mean platelet volume and the diagnosis of acute coronary syndromes. We further aim to evaluate the potential diagnostic efficiency of MPV in the detection of conditions under the spectrum of acute coronary syndromes.

Methods: Adult chest pain patients were tested for CBC examination at the ER of Philippine Heart Center. The specimen was processed for MPV and platelet count using Beckman-Coulter ACT 5Diff hematology auto-analyzer. Patients were grouped into ACS and non-ACS. Independent t-test was used to compare these two groups. Cut-off points of MPV were computed as determined by the area under the curve of ROC curve.

Results: A total of 150 adult patients were examined for MPV and platelet counts. The MPV of patients with ACS was increased at 8.3 fL compared to 7.3 fL in patients not diagnosed with ACS. Cut-off points predictive of ACS based on the area under the curve of the ROC curve revealed a significant test (AOC is equal to 0.868 95% CI (0.812–0.924) $P < 0.0001$). At cut-off point of 8.4 fL, the positive predictive value and specificity for ACS were 100%. MPVs between 7.6 and 7.8 showed satisfactory values of sensitivity, specificity, PPV and NPV. The number of platelets was increased in non-ACS group.

Conclusion: The MPV of chest patients diagnosed with ACS was significantly higher compared to patients not diagnosed with ACS. At cut-off point of 8.4 fL, the value is highly specific and predictive of ACS. The diagnostic accuracy of MPV values is satisfactory between 7.6 fL to 7.8 fL. Platelet count of non-ACS group is increased.

Disclosure of Interest: None declared.

PO026-TUE

Residual platelet function in acs patients treated with prasugrel is lower than in patients treated with clopidogrel but not in patients with hypertension

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Background: Patients with acute coronary syndromes (ACS) receive antiplatelet agents to reduce the risk of recurrent thrombotic events. High residual platelet function (RPF) is associated with increased risk of cardiovascular events and this is particularly evident in those with increased vascular risk factors. Here we have compared RPF in ACS patients during treatment with aspirin and clopidogrel or aspirin and prasugrel in relation to six different vascular risk factors including hypertension.

Aims: To investigate the relationship between RPF and various vascular risk factors in ACS patients one month after start of antiplatelet therapy.

Methods: Platelet function testing was via measurement of P-selectin on platelets using the Aspirin and P2Y12 Test Kits (Platelet Solutions Ltd., UK) where blood samples were stimulated with arachidonic acid or adenosine diphosphate respectively, immediately after blood collection, and stabilized with PAMFix (from PSL); flow cytometric analysis was performed within 9 days of blood collection.

Results:

	No hypertension	Hypertension	P value
Clopidogrel P-selectin MF median [IQR] (n)	606 [392–794] (47)	543 [397–793] (33)	0.913
Prasugrel P-selectin MF median [IQR] (n)	356 [294–455] (43)	463 [323–557] (28)	0.017
P value	<0.001	0.118	

For data obtained using the P2Y12 Test, as expected, RPF was higher in those taking clopidogrel than those taking prasugrel. However, this was only true in patients who did not have hypertension. RPF was significantly higher in those taking prasugrel who had hypertension compared with those without hypertension and values almost reached those seen in the patients taking clopidogrel. No associations with other risk factors were evident using the P2Y12 Test. There were no associations with any risk factors using the Aspirin Test.

Conclusion: Hypertension seems to negate the beneficial effects of prasugrel in some patients with ACS. Platelet function testing might be useful to optimise therapy in this patient group.

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PO027-TUE

Predictors of new onset atrial fibrillation after myocardial infarction

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Background: Atrial fibrillation (AF) may complicate clinical course and treatment strategies of patients after myocardial infarction (MI). Limited data are available on this field.

Aims: To evaluate the predictors of new onset AF after IM in long term follow-up of patients.

Methods: A prospective cohort of patients with a recent IM and no evidence of mitral valve disease admitted to a cardiac rehabilitation between July 2006 and December 2014 was evaluated. Baseline characteristics, risk factors, comorbidities, CHA₂DS₂Vasc score, exercise training risk-class and medications were recorded at admission and new onset AF, heart failure (HF), acute coronary syndrome (ACS), ischemic stroke and death in the follow-up. Data are presented as numbers or percentage for discrete variables and as mean±SD for continuous variables. Chi-square test, student's t-test, uni- and multivariate analyses were used for analyses.

Results: Overall, 319 patients were included in the study: mean age 63 ± 11 years, 79% men. Revascularization procedures were performed in 289 patients: 219 percutaneous technique and 70 surgery. 53%, 37% and 10% of patients presented low, intermediate and high risk class for exercise training, respectively. The average follow-up was

3.2 ± 2.4 years. Twenty-eight (8.8%) patients had AF in the follow-up. The following features were more frequent in patients with new onset AF respect to patients without and confirmed at multivariate analysis: age ≥70 years (60.7% vs. 24.4%), CHA₂DS₂Vasc ≥3 (85.7% vs. 15.5%), HF (32.1% vs. 7.9%), history of AF (28.6 vs. 4.8): $P = 0.002$, $P = 0.039$, $P = 0.005$ and $P = 0.009$, respectively. Complete revascularization was inversely related to AF onset (50% vs. 76%; $P = 0.006$).

Conclusion: In patients with recent MI age ≥70 years, CHA₂DS₂Vasc ≥3, history of HF and AF are the predictors for new onset AF. A complete revascularization is inversely related to the incidence of AF development.

Disclosure of Interest: None declared.

PO028-TUE

Effects of mineralocorticoid receptor antagonists on the risk of mortality, thrombosis and bleeding in patients with secondary hyperaldosteronism: a meta-analysis of randomized controlled trials

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Background: Aldosterone seems to influence the haemostatic system by several mechanisms and by that to increase the risk of thrombosis.

Aims: The objective of this meta-analysis was to assess the impact of mineralocorticoid receptor antagonists (MRAs) on mortality, venous and arterial thrombosis and bleeding in patients with hyperaldosteronism due to any cause.

Methods: We systematically searched PubMed and EMBASE through August 1 2014, without language restriction. We included randomised controlled trials (RCTs) that investigated the effect of MRAs on total and cardiovascular mortality, thrombotic events and bleeding events in patients with hyperaldosteronism due to various causes.

Results: Twenty published RCTs with a total of 17572 patients met the inclusion criteria. Of these RCTs, all reported data on mortality, 15 on cardiovascular mortality, 14 on thrombotic events and 12 on bleeding events. On venous thrombosis, too sparse data was available to be analysed. No heterogeneity was observed among RCTs. Patients treated with MRAs had a decreased risk of total mortality and of cardiovascular mortality compared with controls (odds ratio 0.79; 95% confidence interval (CI), 0.73–0.87; and odds ratio 0.77; 95% CI, 0.70–0.85; respectively, $P < 0.00001$). No difference was observed in the risk of arterial thrombosis and of bleeding events.

Conclusion: MRAs reduce total and cardiovascular mortality in patients with secondary hyperaldosteronism without affecting the risk of myocardial infarction, ischaemic stroke and bleeding. MRAs can be considered as safe regarding their effects on haemostasis.

Disclosure of Interest: None declared.

PO029-TUE

Urinary 11-dehydro-thromboxane B2 is associated with cardiovascular events and mortality in atrial fibrillation patients

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Background: Non-Valvular Atrial Fibrillation (AF) patients show high residual cardiovascular risk despite oral anticoagulants. Urinary 11-dehydro-thromboxane B2 (TxB₂) is associated with an increased risk of cardiovascular events (CVEs), but its predictive value in anticoagulated AF patients is unknown.

Aims: Aim of this was to assess whether urinary 11-dehydro-TxB₂ is a predictor of CVEs in anticoagulated patients with AF.

Methods: Prospective single-center cohort study, including 864 consecutive AF patients. Mean time of follow-up was 30.0 months yielding 2062 person-years of observation. Urinary 11-dehydro-TxB₂ was measured at baseline. The primary end-point was the occurrence of a CVE including fatal/nonfatal myocardial infarction (MI) and ischemic stroke, transient ischemic attack, cardiac revascularization and cardiovascular death.

Results: Mean age of patients was 73.1 years, and 43.6% were female. Median 11-dehydro-TxB₂ levels were 100 [IQR 50–187] ng mg⁻¹ of urinary creatinine. Overall, the anticoagulation control was adequate (63.9% of mean time in therapeutic range). A CVE occurred in 98 (11.3%) patients, 55 were cardiovascular deaths. At baseline, 11-dehydro-TxB₂ levels were higher in patients with a CVE compared to those without (186 [107–400] vs. 98 [52–170], $P < 0.001$). An increased rate of CVEs ($P < 0.001$) and cardiovascular deaths ($P < 0.001$) was observed across tertiles of 11-dehydro-TxB₂. On Cox proportional hazards analysis, third tertile of 11-dehydro-TxB₂ (compared to the first tertile), age ≥75 years, diabetes, history of stroke/TIA and MI/coronary heart disease were significant predictors of CVEs.

Conclusion: Urinary 11-dehydro-TxB₂ levels predict residual risk of CVEs and cardiovascular mortality in AF patients on good anticoagulation control.

Disclosure of Interest: None declared.

PO030-TUE

Association between circulating proteins and corresponding genes expressed in coronary thrombi in patients with acute myocardial infarction

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Background: Scientific efforts have previously been made to map changes in soluble hemostatic and inflammatory mediators during acute myocardial infarction (AMI). To what extent such soluble mediators reflect coronary thrombi gene environment and time elapsed from start of symptoms are unclear.

Aims: To explore whether circulating levels of selected cardiovascular mediators associate with coronary thrombi gene expression and ischemic time.

Methods: In 33 subjects with AMI undergoing revascularisation with percutaneous coronary intervention (PCI), blood samples were collected between 6–24 h after PCI for determination of circulating levels of metalloproteinase 9 (MMP-9), tissue inhibitor of metalloproteinases

1 (TIMP-1), P-selectin, CD40 ligand (CD40L), tissue plasminogen activator (t-PA), plasminogen activator inhibitor 1 (PAI-1), free and total tissue factor pathway inhibitor (TFPI), fibrin degradation product (D-dimer), prothrombin fragment 1 + 2 (F1 + 2), interleukin 8 (IL-8), interleukin 18 (IL-18), fractalkine, pentraxin 3 (PTX3) and monocyte chemoattractant protein 1 (MCP-1). Coronary thrombus RNAs were assessed by RT-PCR with relative quantification. All subjects were participants in the 'Wilhelminenspital Stent Registry' (WSP-STENT) and gave informed consent for study participation.

Results: Soluble P-selectin correlated positively to thrombi P-selectin gene expression ($r = 0.530$, $P = 0.002$). Soluble MCP-1 was higher at short (≤ 4.0 h) vs. long (> 4.0 h) ischemic time (242 vs. 167 pg mL⁻¹, $P = 0.024$). Otherwise, no significant associations were observed.

Conclusion: Circulating mediators related to coronary plaque rupture, platelet activation, coagulation, fibrinolysis and inflammation were mainly unrelated to both coronary thrombi gene expression and ischemic time. These observations indicate a highly local milieu within the site of coronary atherothrombosis, which may be important for selective therapeutic targeting.

Disclosure of Interest: None declared.

PO031-TUE

Vascular smooth muscle cells are responsible for a prothrombotic phenotype of spontaneously hypertensive rat arteries

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Background: The hypothesis that hypertension induces a hypercoagulable state arises from the complications associated with hypertension: stroke and myocardial infarction.

Aims: We determined whether spontaneous hypertension plays a role in the thrombin generating capacity of the vascular wall *in vitro* and modifies the thrombotic risk *in vivo*.

Methods: Spontaneously hypertensive rats (SHR) were compared with Wistar rats. Thrombin generation was monitored using calibrated automated thrombography in a rat pool plasma in which aortic rings had been added. The effect of an antihypertensive drug was tested. Thrombosis was triggered by ferric chloride (FeCl₃) application to the carotid artery.

Results: The addition of thoracic aortic rings of SHR to a Wistar or SHR plasma pool resulted in a greater increase in thrombin generation compared to equivalent rings from Wistar. This increase occurred in 12 but not 5 week-old rats and was prevented by an angiotensin II-converting enzyme inhibitor, indicating that established hypertension is required to induce increased thrombin generation within the vessel wall. In cell culture, thrombin formation was higher on aortic smooth muscle cells (SMCs) from SHR than on those from Wistar whereas no difference was observed for endothelial cells. Exposure of negatively-charged phospholipids was higher on SHR than on Wistar rings as well as on cultured SMCs. SHR SMCs were more sensitive to thrombin-induced proliferation than Wistar SMCs. This effect was totally abolished by a protease-activated receptor 1 inhibitor. *In vivo*, 12 week-old SHR exhibited accelerated FeCl₃-induced thrombus formation in carotid arteries and the resulting occlusive thrombi were disaggregated by blockade of glycoprotein Iba-von Willebrand factor interactions.

Conclusion: The prothrombotic and proliferative phenotype of the SHR vessel wall was due to the ability of SMCs to support greater thrombin generation and to proliferate via PAR-1-dependent thrombin signaling.

Disclosure of Interest: None declared.

PO032-TUE

Platelet ERK5 regulates thrombosis and myocardial infarct extension via matrix metalloproteinases

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Background: Dysregulated platelet activation in an ischemic microvascular environment may play a role in myocardial infarction (MI). We discovered that ERK5, a nuclear protein which is reactive oxygen species (ROS)-activated in others cells, is abundantly present in platelets.

Aims: We investigated whether ERK5 could regulate platelet activation in isolated platelets *in vitro*, and using three *in vivo* models of thrombosis.

Methods: Human and mouse platelets were stimulated with surface agonists or ROS (H₂O₂ or 5% O₂). ERK5 activity was assessed by immunoblotting. Platelet activation was assessed via fluorescent-activated cell sorting (FACS) for P-selectin or activated GPIIb/IIIa. Intravascular thrombus (pulmonary embolus) or mesenteric thrombus (oxidative injury) formation was assessed by *ex vivo* fluorescent imaging and *in vivo* intravital microscopy, respectively. MI was performed in wild-type (WT) and in platelet specific ERK5 deficient (ERK5^{-/-}) mice by LAD coronary artery ligation. Left ventricular (LV) function was determined by echocardiography. Matrix metalloproteinase (MMP) activity was determined by in-gel zymography

Results: Human and platelet ERK5 was activated by ROS. Murine *in vivo* thrombosis was regulated by platelet ERK5 only if the injury involved oxidative stress. MI in mice promoted sustained platelet activation over one week in an ERK5-dependent manner. Following MI, platelet ERK5^{-/-} mice had similar bleeding times but less reactive platelets, less platelet MMP activity, decreased thromboxane production, attenuated MMP activity in the LV, less remodeling with smaller infarcts, and enhanced myocardial systolic performance.

Conclusion: ERK5 is an ischemic sensor in platelets which regulates ongoing platelet activation after MI as well as myocardial tissue remodeling. These observations may explain ischemic microvascular aberrations like the no-reflow phenomenon following percutaneous coronary intervention, suggesting a novel pharmacologic target which avoids bleeding complications.

Disclosure of Interest: None declared.

PO033-TUE

Elevated arterial stiffness and thrombin generation in patients with end-stage renal disease undergoing hemodialysis

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Background: Cardiovascular diseases, including increased arterial stiffness, are the main cause of mortality in end-stage renal disease (ESRD). Anti-platelet drugs are used to reduce platelet-vessel wall interactions and subsequent thrombin generation and fibrin clot formation.

Aims: To test whether increased arterial stiffness may contribute to a hypercoagulable state in ESRD.

Methods: Fifty five ESRD patients were explored before dialysis and compared to 10 controls. Arterial stiffness was measured with high precision echotracking devices. Platelet aggregation was monitored by turbidimetry and thrombin generation (TG) by calibrated automated thrombography.

Results: Among the 55 patients (mean age: 71 ± 13 years; blood pressure: 141 ± 22/73 ± 12 mmHg), 29 received anti-platelet drugs.

Aortic PWV is high ($12.0 \pm 3.1 \text{ m s}^{-1}$) by comparison with recently published reference values. Platelet aggregation was reduced in patients receiving anti-platelet drugs. TG without activated protein C (APC) was not different between patients and controls. In platelet rich plasma, TG was less sensitive to APC in patients without anti-platelet drugs. By contrast, no difference was found in platelet-poor plasma or in patients with anti-platelet drugs compared to controls. TG was positively correlated with carotid stiffness. Endothelial microvesicles (MVs), plasma von Willebrand factor and tissue factor pathway inhibitor were positively correlated with aortic stiffness as well as tissue factor-positive platelet or endothelial MVs. Anti-platelet therapy did not influence endothelial dysfunction and arterial stiffness.

Conclusion: Our study demonstrates that ESRD increases arterial stiffness and procoagulant factors and decreases endothelium function and the APC pathway. They suggest that arterial stiffness may play a causal role in the pro-thrombotic phenotype. In ESRD, arterial stiffness and endothelial function are insensitive to anti-platelet treatment, in contrast to APC downregulation of TG.

Disclosure of Interest: None declared.

PO034-TUE

Thrombogenic state after vascular surgery and perioperative cardiovascular events

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Background: Cardiovascular events (CVE) are the most limiting prognostic factor after vascular surgery. The underlying mechanism is thrombosis in patients with high atherosclerotic burden.

Aims: To identify baseline coagulation markers related to CVE and to analyze the behavior of those markers after surgery.

Methods: Thrombin generation, assessed as endogenous thrombin potential (ETP) and ETP ratio (with-to-without thrombomodulin), and platelet aggregation in response to arachidonic acid (Aggr) were evaluated before and after surgery, for 183 patients under aspirin use. Patients were monitored for CVE detection: acute coronary syndromes, isolated troponin elevation, stroke, reoperation due to thrombosis and cardiac death. Our ethics committee approved the study and patients provided informed consent.

Results: The only baseline coagulation marker independently related to CVE was Aggr, so that patients in the 4th quartile had a 2.42 fold increased risk ($P = 0.034$). After exclusion of patients without post-operative test and 53 who received hemoderivatives, we analyzed the behavior of ETP, ETP-ratio and Aggr for 110 patients. There was a marked increase in ETP: $648 \text{ NM} \cdot \text{min} \pm 457 \times 887 \pm 494$; $P < 0.001$, ETP-ratio $0.43 \pm 0.25 \times 0.61 \pm 0.28$; $P < 0.001$, and a significant decrease in Aggr: $5.34 \pm 5.69 \times 3.38 \pm 5.30 \text{ } \Omega$ ($P < 0.001$) and in platelet count $1000: 229 \pm 76 \times 194 \pm 73$; $P < 0.001$. We calculated the individual variable differences (Δ) after and before surgery, and then we compared those means between patients with and without CVE. There was no difference in Δ ETP ($P = 0.87$), Δ ETP-ratio ($P = 0.61$) or Δ platelet count ($P = 0.57$), but patients with CVE had a greater Δ Aggr (-4.27×-1.37 ; $P = 0.04$).

Conclusion: There is a pro-thrombotic state triggered by the surgical stress. The apparent contradiction of increase in thrombin generation and decrease in Aggr suggests platelet consumption, which is greater when CVE occurs. Aspirin responsiveness before surgery is more important than net thrombin generation for the occurrence of perioperative CVE.

Disclosure of Interest: None declared.

PO035-TUE

Clinical features of vascular events in patients with al amyloidosis

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Background: AL amyloidosis can cause various vascular events, which are related to vascular amyloid deposition or thromboembolism. Despite their clinical importance, only few reports have been published on the manifestations of vascular events in these patients

Aims: We aimed to identify the incidence of vascular events in patients with amyloidosis and find out the characteristics of patients who experience the vascular events.

Methods: We retrospectively reviewed medical records of patients including various imaging study results such as angiography, Doppler sonography, and magnetic resonance imaging to find out clinically significant vascular stenosis and thromboembolism.

Results: Between January 2001 and September 2013, 86 patients (male/female=50/36) with biopsy-proven AL amyloidosis were included from 3 hospitals. The median age was 58 years (range, 24–81). Between the onset of the 1st clinical presentation of amyloidosis and the time of pathologic diagnosis, 5 patients (5.8%) experienced 5 events of vascular stenosis. During a median follow-up of 19.6 months (range, 0.2–87.3), 20 events of vascular stenosis developed in 16 patients (23.3%) and 6 events of thromboembolism in 6 patients (7.0%). Overall, after the 1st clinical presentation of amyloidosis, 24 patients (27.9%) experienced vascular events: 17 patients (19.8%) experienced 18 events of vascular stenosis including cerebrovascular disease (15), coronary artery disease (3), and 6 patients (7.0%) experienced 6 events of thromboembolism. The number of patients who had both vascular stenosis and thromboembolism was 3 (3.4%). Patients with renal involvement suffered significantly more from vascular events than the other ones (39.5% vs. 16.3% $P = 0.028$). The median overall survival (OS) from pathologic diagnosis was 31.0 months (95% CI, 7.7–54.3). The 1 yr OS after the onset of vascular event was 55%.

Conclusion: The incidence of vascular events was substantial in patients with AL amyloidosis, especially in patients with renal involvement of amyloidosis.

Disclosure of Interest: None declared.

Atherosclerosis II

PO036-TUE

Simultaneous observation of peripheral mononuclear cell and plasma TF's expression for prevention and treatment of ischemic cardiocerebrovascular diseases

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Background: The role of cell-derived TF in the formation of pathologic thrombosis has aroused increasing attention. Mononuclear cells (Mo) are blood cells that were earliest found to express TF, and the only generally accepted blood cells that can intrinsically synthesize TF. Whether measurement of MoTFmRNA change alone can determine

the up-regulation of TF expression remains unclear. In this study, we will demonstrate it.

Aims: Simultaneously observe the expression of mononuclear cells (Mo) and plasma tissue factor (TF) in patients with ischemic cerebrovascular diseases to explore their clinical implications.

Methods: To compared with those of 61 healthy controls, MoTFmRNA and plasma TF antigen (TFag) were measured dynamically by RT-PCR and ELISA in 76 patients with acute myocardial infarction (AMI) and 46 patients with acute ischemic stroke (AIS).

Results: Compared with the control group, MoTFmRNA and plasma TF increased simultaneously during the acute stage in other groups, especially in AIS group, showing a good correlation between them ($P < 0.01$). At 3 weeks after onset, both of them were still significantly higher than in control group ($P < 0.01$ and $P < 0.05$). At 3 months after onset, MoTFmRNA began declining in AMI and AIS groups, though they were still significantly higher than control group ($P < 0.05$). In AIS group, MoTFmRNA level at 3 months after onset was significantly lower than at the acute onset stage ($P < 0.05$), while plasma TF in AMI and AIS groups decreased significantly to a level that was not significantly different with control group ($P > 0.05$), though it was still significant different from that during acute onset stage ($P < 0.05$).

Conclusion: Dynamic monitoring of MoTFmRNA and plasma TF at different time points after acute onset has important clinical implications in the prevention and treatment of arterial thrombotic diseases.

Disclosure of Interest: None declared.

PO037-TUE

Total chemical synthesis of covalent Rantes-PF4 heterodimers to study initiation and progression of atherosclerosis

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Background: Atherosclerosis is caused by chronic inflammation of the arterial wall through recruitment of mononuclear cells, a process that is exacerbated by platelet-dependent deposition of chemokines RANTES and Platelet Factor 4 (PF4) on the inflamed endothelium. It was found that non-covalent heterodimer formation of RANTES and PF4 promotes monocyte recruitment to the vascular wall and might provide a clinical target for prevention of atherosclerosis and arterial thrombosis.

Aims: To study RANTES-PF4 heterodimer dependent monocyte-endothelium bridging, synthetic access to- and biologic characterization of covalently linked RANTES-PF4 heterodimers is needed.

Methods: The site of coupling of the two proteins was based on molecular dynamics and NMR structural analysis that showed interactions in the N-termini of the free chemokines. However, as RANTES interacts with its receptor via the N-terminal amino acids it was hypothesized that a coupling of the two N-terminal amino acids would not lead to an active dimer. Positions for covalent linkage of the two proteins were postulated that do not affect chemokine function. Both PF4 and RANTES were synthesized in three parts by tBoc solid phase peptide synthesis and ligated using native chemical ligation. Heterodimers were tested in an endothelial monocyte arrest assay under flow.

Results: The functionalized proteins were folded and subsequently ligated using an oxime bond to result in the RANTES-PF4 heterodimer. Both a dimer with expected biological activity and an inactive control were synthesized. Surfaces treated with the covalent heterodi-

mer show a significant increase in monocyte arrest as compared to non-covalent heterodimer. Currently murine heterodimers are tested in an *in vivo* mouse model to confirm the exacerbation of atherosclerosis by RANTES-PF4 heterodimer.

Conclusion: Covalent RANTES-PF4 heterodimers were synthesized and were shown to increase endothelial monocyte arrest compared to non-covalent heterodimers.

Disclosure of Interest: None declared.

PO038-TUE

Endothelial deletion of thioredoxin-interacting protein preserves mouse arteries from aging induced by metabolic disorders

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Background: Recent studies have implicated the thioredoxin (TRX) system and its endogenous inhibitor, TRX-interacting protein (TXNIP), in endothelial homeostasis and in a decrease of aortic distensibility leading to cardiovascular disorders in aging, mostly due to loss of antioxidant systems. We postulated that endothelial TXNIP deletion might provide protection from arterial aging induced by metabolic stress, redox imbalance, inflammation, and prothrombotic state.

Aims: To investigate the effects of endothelial TXNIP deletion on arterial damage related to aging induced by metabolic disorders.

Methods: We generated endothelial TXNIP knock-out (EC-TXNIP KO) mice on the Cdh5-cre background and subjected them to a 3 month high protein-low carbohydrate (HP-LC) diet, known to induce metabolic disorders and vascular damage mimicking accelerated aging. The impact of EC-TXNIP deletion was evaluated with regard to glucose tolerance, lipemic parameters and cardiovascular status using Doppler echography, by comparison with wildtype and KO mice fed a HP-LC diet. Oxidative stress and aortic inflammation were evaluated by immunocytochemistry labeling, dihydroethidium staining, PAI-1 antigen level and RT-PCR. Platelet activation was measured by flow cytometry.

Results: Compared with littermate controls, EC-TXNIP KO mice were less subject to endothelial dysfunction due to metabolic stress. This protective effect was associated with diminished endothelial NADPH oxidase expression and reduced aortic superoxide levels. A decrease of endothelial IL-1 β expression in the aorta and a lower PAI-1 secretion was observed showing a reduced pro-inflammatory state induced by metabolic stress in EC-TXNIP KO mice. The EC-TXNIP KO mice were also protected from platelet hyperactivation, distensibility impairment and heart failure due to metabolic stress.

Conclusion: These findings demonstrate the key role of TXNIP in cardiovascular impairments induced by metabolic stress, making TXNIP a potential therapeutic target.

Disclosure of Interest: None declared.

PO039-TUE

High lipoprotein (A) levels are associated with coronary calcium score in the montignoso study population

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Background: Different studies showed a causal role of lipoprotein (a) [Lp(a)] in the pathogenesis of atherosclerosis and cardiovascular diseases.

Aims: Aim of this study was to evaluate the association between Lp(a) levels and coronary artery calcium score (CACS) in the prospective general population study 'Montignoso Study'.

In 298 subjects (M 224/F 74 median age 69) we studied CACS Agatston score (CACS_{Ag}) Agatston score, Calcium Volume Score (CVS) and Lp(a) levels.

Methods: We used an isoform-insensitive immunoreactivity to apo(a) method (Randox). High Lp(a) levels were defined values ≥ 500 mg L⁻¹.

Results: Subjects with CACS_{Ag}>100 ($n = 149$) had significantly higher Lp(a) levels than subjects with CACS_{Ag} ≤ 100 ($n = 149$) [124.0 (63.0–384.0) vs 101.0 (55.0–183.5) mg L⁻¹, $P = 0.015$]. Similarly, Lp(a) levels in subjects with CVS>100 were significantly higher than in subjects with CVS ≤ 100 [124.0 (61.5–382.5) vs 97.0 (56.1–185.3) mg L⁻¹, $P = 0.023$].

A higher percentage of subjects with Lp(a)>500 mg L⁻¹ was observed in CACS_{Ag}>100 than in CACS_{Ag} ≤ 100 subjects (20.8% vs 8.7%, $P = 0.003$).

At regression logistic analysis adjusted for age, sex, cardiovascular risk factors Lp(a) levels ≥ 500 mg L⁻¹ were significantly associated with CACS_{Ag}>100 [OR=2.56 (95% IC 1.25–5.28), $P = 0.011$]. Similarly, the logistic regression analysis showed that Lp(a) levels ≥ 500 mg L⁻¹ were significant predictors of CVS>100 [OR=2.50 (95% IC 1.22–5.16), $P = 0.013$].

Conclusion: Our results showed a significant association between high levels of Lp(a) and CACS, suggesting a significant role of Lp(a) in atherosclerosis.

Disclosure of Interest: None declared.

PO040-TUE

Association of gamma-glutamyl transferase to the premature coronary artery disease in young Asian Indians

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Background: Accumulating evidence from epidemiological studies suggests that higher gamma Glutamyl Transferase (GGT) level in blood is associated with incident cardiovascular disease (CVD) including atherosclerosis and has prognostic importance. However, the association of GGT levels to the premature coronary artery disease in Asian Indian population has not been evaluated.

Aims: To evaluate risk prediction value of GGT in premature CAD subjects.

Methods: In this present study, 240 (120 unaffected and 120 affected) out of 2318 subjects of phase-I, from Indian Atherosclerosis Research Study (IARS) were selected. Markers assayed were GGT, hsCRP, lipids, sPLA2, neopterin, myeloperoxidase, IL6, Cystatin-C and Lp(a). Statistical analysis was performed using SPSS-ver17 software.

Results: The plasma GGT levels in these subjects showed positive correlation with continuous variables such as waist circumference, triglycerides, Neopterin levels and cross sectional correlation with binary variable smoking but not with alcohol habit. Our findings suggests that the GGT alone had an odds ratio (OR) of 1.909 (95%CI-1.036–3.517; $P = 0.038$) for the third tertile which improved to 2.104, (95% CI-1.063–4.165; $P = 0.033$) after adjusting to conventional risk factors (CRF). The logistic regression analysis showed that GGT alone had a odds ratio of 4.94 (95%CI 1.015–24.095; $P = 0.048$) which improved to 5.636 (95%CI 1.004–31.642; $P = 0.049$) and 7.492 (95%CI 1.221–45.979; $P = 0.030$) after addition of Lp(a) and adjustment with CRFs respectively. The C-statistic analysis showed an significant (De-Long $P < 0.001$) improvement of AUC from 0.647 ($P < 0.001$) for CRFs alone to 0.711 ($P < 0.001$) for final GGT plus Lp(a) model.

Conclusion: In summary, the elevated plasma GGT levels potentially indicate increased oxidative stress and risk of developing premature CAD. Hence these findings could be potentially used in the risk stratification of premature CAD after further evaluation.

Disclosure of Interest: None declared.

PO041-TUE

Coronary artery disease severity is a marker of the anti-inflammatory response to statin therapy

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Background: Statins are an essential therapy in the management of coronary artery disease (CAD). Based on the 2013 cholesterol guidelines, all patients with CAD should be on statins. There is also a growing concern that the new guidelines would lead to statin overuse.

Aims: To evaluate the relation of detailed lipid profile and coronary artery disease severity and inflammation during statin therapy

Methods: In the Multi-Analyte, Thrombogenic, and Genetic Markers of Atherosclerosis study, we enrolled patients ($n = 585$) with suspected CAD undergoing elective coronary angiograms. CAD severity was categorized angiographically as none-minor CAD (<20% diameter stenosis), non-significant CAD (20%–75%), and significant CAD (>75%) of any major coronary vessel. Blood and urine samples were obtained immediately prior to cardiac catheterization. Detailed lipid profiling was performed using VAP cholesterol test, urinary 11-dehydrothromboxane B₂ (dTxB₂) was tested using Aspirinworks™, and AtherOx testing was done using immunoassay.

Results: Nineteen percent patients had minor CAD, 19% had non-significant CAD and 62% had significant CAD. Overall, 24% patients with CAD were not on statin therapy. 50% of patients with none-minor CAD were on statin therapy. Statin therapy was associated with lower total cholesterol, total LDL, LDL₁, LDL₂, LDL₃, Ox-LDL, total VLDL, Apo B100/A1 ratio ($P \leq 0.04$). Statin therapy was associated with an anti-inflammatory effect only in patients with significant CAD. Irrespective of statin therapy, only total HDL was lower in patients with significant CAD ($P < 0.05$).

Conclusion: Although statin therapy improves the lipid profile irrespective of CAD severity it appears to decrease inflammation primarily in patients with significant CAD. These data support the recent guidelines for statin treatment according to patient risk. Urinary dTxB₂ may be a novel tool to enhance identification of patients for statin therapy.

Disclosure of Interest: None declared.

PO042-TUE

Effects of 12 months exercise intervention on adipose tissue expression of chemokines in patients with type 2 diabetes and stable coronary artery disease: a substudy of a randomized controlled trial (RCT)

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Background: Low grade chronic inflammation plays a role in atherosclerosis and type 2 diabetes (T2DM), and the inflammatory activity in adipose tissue is considered important.

Aims: We aimed to investigate if 12 months regular exercise in patients with both T2DM and coronary artery disease (CAD) reduces the genetic expression of the proinflammatory markers fractalkine (CX3CL1), its receptor (CX3CR1) and MCP-1 in subcutaneous adipose tissue. Circulating levels of fractalkine and MCP-1 were also investigated.

Methods: Patients were included in a RCT investigating the effects of exercise on atherosclerosis progression and glucose control in patients with CAD and T2DM. The Regional Ethics Committee approved the study and patients gave written informed consent to participate. Patients were randomized 1:1 to exercise training (combined aerobic and strength training) or control. At inclusion and after 12 months, fasting venous blood samples and a subcutaneous adipose tissue sample were taken. Circulating fractalkine and MCP-1 were determined by ELISA. RNA was extracted from the adipose tissue, and fractalkine, CX3CR1 and MCP-1 expression examined by RT-PCR.

Results: Analyses were performed in 114 patients who completed the study and adhered to the intervention. No between group differences in gene expression levels of the markers in adipose tissue were found at both time points, and also no between group differences in changes from baseline. Circulating fractalkine increased from (median (IQR)) 431(348–556) pg mL⁻¹ to 445(345–577) pg mL⁻¹ after 12 months in the exercise group ($P = 0.044$), significantly more compared to controls, $P = 0.042$. MCP-1 was not significantly changed in either group. **Conclusion:** No significant reductions in subcutaneous adipose tissue expression or circulating levels of the selected markers after 12 months regular exercise training in patients with both CAD and T2DM were demonstrated. This might reflect the selection of biomarkers, the intensity of the intervention or the severity of CAD and T2DM.

Disclosure of Interest: None declared.

PO043-TUE

Atherosclerotic cardiovascular mortality in gouty arthritis and rheumatoid arthritis: is it associated with mean platelet volume and neutrophil/lymphocyte ratio

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Background: Mean platelet volume (MPV) was reported to be a biomarker for platelet activation; neutrophil/lymphocyte ratio (NLR) might also be related to development of atherosclerotic cardiovascular mortality (ACVM).

Aims: We evaluated MPV and NLR in gouty arthritis (GA) and rheumatoid arthritis (RA) patients. We also analyzed their relationship between ACVM.

Methods: 122 GA (96 M, 26 F, mean age: 64.6), 82 RA (40 M, 42 F, mean age: 62.1) patients and 61 healthy controls (34 M, 27 F, mean age: 64.3) were included. The clinical and laboratory data were obtained from medical charts. ESR, CRP, MPV and NLR were

recorded at the time of diagnosis and after one month of therapy. Duration of follow-up and atherosclerotic events were recorded from medical records.

Results: MPV in GA (8.5 ± 1.5) and RA (7.98 ± 0.1) groups were significantly lower than in healthy controls (9.8 ± 1.5) ($P < 0.001$). NLR in healthy controls (1.9 ± 0.7) was significantly lower than in GA (3.7 ± 2.3) and RA (3.7 ± 2.5) groups ($P < 0.001$). After treatment, MPV decreased significantly in GA and RA groups ($P < 0.001$). NLR also decreased in GA and RA patients ($P < 0.001$). 9 patients with GA and 12 patients with RA died from ACV complications during follow-up. GA patients with ACVM were older and had more frequent hypertension, higher MPV ($P < 0.05$). In multivariate analysis, MPV was an independent poor prognostic factor for ACVM (OR: 50, 95%CI: 1.01–642, $P = 0.05$). RA patients with ACVM were more frequently males, older, had higher platelet count and hypertension. NLR and uric acid levels were significantly higher in ACVM group ($P \leq 0.01$). Multivariate analysis in RA group revealed that male sex (OR: 66.9, 95% CI: 1.03–432, $P = 0.04$) and NLR (OR: 1.9, 95%CI: 1.2–3, $P = 0.01$) were independent factors for ACVM.

Conclusion: MPV and NLR decreased after treatment all GA and RA groups. MPV was a poor prognostic biomarker for ACVM in both univariate and multivariate analysis in GA. NLR was an independent prognostic factor for ACVM in RA patients.

Disclosure of Interest: None declared.

Blood coagulation tests II

PO044-TUE

Evaluation of the laboratory method in pregnant women with complicated obstetric history for haemostasis monitoring

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Background: The problem of habitual miscarriage is one of the most difficult medical and social problems.

Aims: The aim of the study was to evaluate the possibility of using the method of the spatial growth dynamics of fibrin clot (Thrombodynamics) in pregnant women with complicated obstetric history (COH).

Methods: The study involved 21 pregnant women with COH. The group 1 consisted of women who were at 13–20 weeks of gestation, group 2–21–27 weeks. On the «Registrar of Thrombodynamics T-2» was determined to delay the growth of the fibrin clot Tlag (reference values of 0.8–1.5 min), the clot growth velocity V ($20.5\text{--}30 \mu\text{m min}^{-1}$). Tests APTT and D-dimer (as reference values was used standards for reagents Stago) were made on coagulometer Sta Compact (Roche). The median (Me) and 1 and 3 quartiles (Q1-Q3) were used to describe the quantitative data.

Results: The age of women in group 1 was 32.5 years (31–35), in group 2–30 years (28–35). Parameters of Thrombodynamics, APTT and D-dimer were within the reference values against the backdrop of ongoing antithrombotic therapy. The level of D-dimer was significantly higher ($P < 0.001$) in group 2. In group 1 and 2 V was 25 (23.7–26.3), 27.1 (24.6–29.6); Tlag – 1 (0.8–1.2), 1.1 (0.9–1.7); APTT – 34 (29–38), 34 (33–38); D-dimer – 0.49 (0.4–0.93), 0.97 (0.65–1.33), respectively.

Conclusion: Test Thrombodynamics along with D-dimer can be used for monitoring of hemostasis in pregnant women with COH

Disclosure of Interest: None declared.

PO045-TUE

Effects of low and high levels of non VKA oral anticoagulants on a comprehensive panel of routine and specialty coagulation tests

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Background: Non VKA oral anticoagulants (NOACs) are known to have various effects on hemostasis assays depending on the test principle and on the reagent used.

Aims: The aim of this study is to evaluate the impact of different levels of dabigatran, rivaroxaban and apixaban on a wide range of routine and specialty tests.

Methods: Pooled normal plasma was spiked with dabigatran, rivaroxaban or apixaban at concentration ranging from 0–500 or 0–800 ng mL⁻¹. Samples were immediately frozen at -70°C.

Stago routine tests (PT, Owren PT, aPTT, Thrombin Time (TT), Clauss Fibrinogen) and specialized assays including endogenous and exogenous factors assays, thrombophilia (PC, PS, APCr, anti-IIa based AT assay, lupus anticoagulant (LA) testing), fibrinolysis markers (D-dimer, FM, FDP) were performed on the STA-R® Evolution analyzer.

Results: At 30 ng mL⁻¹, rivaroxaban and apixaban show no significant effect on all tests. In contrast, the effect of dabigatran is clearly demonstrated on TT, PS activity and APCr, while aPTT tests are moderately affected.

At 250 ng mL⁻¹, apixaban shows a moderate effect (only factor V, VIII, IX, XI, XII and APCr are affected). On the opposite, rivaroxaban impacts all the tests except Fibrinogen, TT and AT. At 200 ng mL⁻¹, dabigatran strongly affects aPTT, TT, thrombophilia and endogenous factor assays.

No significant effect is observed on the Clauss Fibrinogen, Owren PT tests and fibrinolysis assays from any drug spiking.

In addition, there is no false positive with the three NOACs at 30 ng mL⁻¹ when using LA confirmatory tests, even though some of the screening tests are affected. At higher concentrations, the only NOAC tested inducing false positive results was rivaroxaban when the DRVV screening and confirmatory tests were used.

Conclusion: While prolonged routine tests (PT, aPTT, TT) may alert on the presence of a NOAC, interferences of NOACs on specialty tests should be well determined during the assay selection and validation process in order to interpret results in the most accurate fashion.

Disclosure of Interest: S. Perroche Employee of: Stago, N. Barat Employee of: Stago, F. Depasse Employee of: Stago, F. Nicham Employee of: Stago, A. Magdelaine Employee of: Stago, D. Courtois Employee of: Stago, T. Hervé Employee of: Stago, J. Beltran Employee of: Stago.

PO046-TUE

FVIII sensitive automated thrombin generation test of canine plasma

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Background: The calibrated automated thrombogram (CAT) assay is a highly valuable tool for testing the effectiveness of anti-haemophilic drugs (AHD) *in vitro* and *ex vivo* in humans. Canine models of haemo-

philia are important for pre-clinical testing of AHDs, but commercially-available human CAT assays are not sensitive to factor VIII (FVIII) activity in canine plasma. Consequently the available methods do not reflect the FVIII activity or the clinical phenotype, and are not able to predict the effect of AHD in canine haemophilia A (HA).

Aims: To develop a CAT method that is highly sensitive to FVIII activity in canine plasma.

Methods: Thrombin generation was initiated by co-dispensing activated partial thromboplastin test reagent, Ca⁺⁺ and a thrombin-sensitive substrate into canine plasma samples, and measured utilizing an automated fluorescence reader. A canine HA plasma pool was spiked with normal canine plasma, or recombinant canine- or human FVIII ranging from 0.001–1 IU mL⁻¹ FVIII activity (final). Precision of the method was determined using a normal canine plasma pool.

Results: At baseline, no, or extremely low, thrombin generation was observed in canine HA plasma. Dose-dependent thrombin generation was observed in canine HA plasma spiked with 0.001–1 IU mL⁻¹ canine FVIII. As little as 0.001 IU mL⁻¹ canine FVIII activity, produced significantly increased thrombin generation parameters in HA plasma. Intra- and inter-assay precision analysis of four CAT parameters revealed coefficients of variation spanning 2–5% (nested analyses).

Conclusion: We have established the first canine optimized CAT assay that, in contrast to commercial available assays, is sensitive to canine FVIII activity. The method is sensitive to very low levels of FVIII, and will potentially be a valuable tool for monitoring treatment of HA dogs with FVIII replacement therapy, bypassing agents, or gene therapy.

Disclosure of Interest: D. E. Madsen Employee of: Novo Nordisk A/S, Denmark, T. Nichols: None Declared, E. Waters Employee of: Novo Nordisk A/S, Denmark, P. Holm Employee of: Novo Nordisk A/S, Denmark, E. Merricks: None Declared, A. Wolberg: None Declared, B. Wiinberg Employee of: Novo Nordisk A/S, Denmark.

PO047-TUE

Different centrifugation conditions do not interfere with D-dimer results

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Background: Preanalytics including centrifugation is crucial for coagulation testing. Important is the achievement of platelet-poor plasma (CLSI H21-5 guideline). However, no specific instruction is provided: centrifugal speed/duration must be established by the laboratory; most common conditions are 1500 g for no less than 15 min. These conditions can translate into unacceptable turnaround time (TAT) in emergency setting. However it is elsewhere reported that D-dimer (DD) (semiquantitative latex agglutination test) levels are not affected by centrifugation conditions or platelet contamination.

Aims: To validate higher speed/shorter centrifugation conditions for DD testing using a DD assay validated for exclusion of venous thromboembolism (VTE).

Methods: Blood (9 volumes) collected in a standard of care in 2 different 2.7 mL 0.109 M trisodium citrate (1 volume) tubes (Vacutainer, Becton-Dickinson, Plymouth, United Kingdom) from 50 patients. Centrifugation at room temperature (ROTINA 380 centrifuge, Hettich, Tuttlingen, Germany) within 1 h under 2 different conditions before DD testing using STA®-Liatest® D-Di on STA®-R analyzer (Stago, Asnieres sur Seine, France): (i) 15 min., 2500 g (package insert recommendation) and alternatively (ii) 5 min., 2026 g.

Results: To significant differences in overall DD results between the 2 different centrifugation conditions.

Mean DD values similar in both arms (1.15 vs. 1.14 mg mL⁻¹).

Concordance of results for VTE exclusion (validated cut-off: $0.5 \mu\text{g mL}^{-1}$) in all samples irrespective of centrifugation conditions, except for 2 samples: 0.59/0.45 and 0.50/0.45 mg mL^{-1} respectively.

Conclusion: Alternative centrifugation conditions have no clinically significant impact on DD level. Discrepancies observed for DD values close to cut-off are likely related to assay expected reproducibility according to CLSI guideline H59-A.

Alternative centrifugation conditions allow faster TAT that can improve patient's management in emergency care.

Disclosure of Interest: C. Aguilar Speaker Bureau of: Speaker honoraria from Diagnostica Stago, S. Savigny Employee of: Diagnostica Stago

PO048-TUE

Thrombin generation in the normal population – flattened thrombin generation curves associated with poor lipid profile and male sex

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Background: Thrombosis and cardiovascular disease are major causes of morbidity and mortality. Unfortunately, there remains no laboratory tests to evaluate this risk. Global coagulation assays such as calibrated automated thrombogram (CAT) maybe a better surrogate measure. However, it is important to first understand the impact of these assays in the normal population, particularly the impact of age and sex.

Aims: Determine thrombin generation parameters in normal controls.

Methods: All normal controls were evaluated with routine laboratory tests to exclude underlying thrombosis risk factors including full blood examination, thrombophilia screen, von Willebrand studies. Individuals on anticoagulants, antiplatelet agents, hormone therapy or on treatment for cardiovascular risk factors were excluded. All samples were double centrifuged at 2500 G and frozen at -80°C within 2 h of collection. Samples were analysed using the CAT using standard 5 pmol reagent (Stago).

Results: 32 normal controls (20 F, 12 M) with median age of 45 (range: 24–79) years were recruited. Thrombin generation parameters varied significantly within this controlled population group and there was no correlation with age ($r^2 = 0.059$). Thrombin generation was higher in females (ETP: 1418 nm vs. 1282 nm, $P = 0.032$). There were 2 distinct patterns of thrombin generation curves – the flattened curve, with lower thrombin peak and velocity index but with a relatively preserved ETP, is more common in males (88% vs 23%, $P < 0.001$) and associated with higher LDL (3.9 vs 3.0, $P = 0.07$), lower HDL, (1.16 vs 1.95, $P < 0.001$) and higher triglyceride levels ($P < 0.001$).

Conclusion: Thrombin generation does not increase with age. Females have higher thrombin generation which maybe related to underlying hormonal status. There appears to be two distinct thrombin generation curves, with the flattened curve associated with male sex and poor lipid profile. This may represent increased cardiovascular risk and will require further evaluation.

Disclosure of Interest: None declared.

PO049-TUE

The rivaroxaban-calibrated anti-fxa assay can reliably identify presence of rivaroxaban in plasma

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Background: One of the most attractive features of oral direct inhibitor of activated coagulation factor X (FXa) rivaroxaban is its administration without dose adjustment based on laboratory tests. In certain clinical circumstances however, e.g. when a patient is bleeding or requires urgent surgery, the evaluation of the impact of rivaroxaban on hemostatic tests may guide further therapeutic decisions.

Aims: To determine the performance of 6 laboratory tests in patients receiving therapeutic doses of rivaroxaban.

Methods: We investigated 28 patients, aged 20–70 (44.5 ± 13.0), who were receiving 20 mg of rivaroxaban daily for at least 10 weeks after an episode of deep vein thrombosis, and 41 controls who did not receive any medication. The renal function was normal in all subjects. Plasma samples from patients were obtained immediately prior to the next rivaroxaban dose (trough) and 4 h following oral dosing (peak). The results of the following tests were analysed: prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen concentration, anti-FXa concentration and thrombin generation peak.

Results: There was statistically significant ($P < 0.05$) difference between peak and trough values (mean $\pm$ sd) for all tests, but highest for PT (s), APTT (s), TGp (nm) and anti-Xa (ng mL^{-1}): $\text{PT}_{\text{peak}}/\text{PT}_{\text{trough}}$ 1.23 ± 0.12 (95%CI 1.18–1.27), $\text{APTT}_{\text{peak}}/\text{APTT}_{\text{trough}}$ 1.25 ± 0.11 (95%CI 1.21–1.29), $\text{TGp}_{\text{trough}}/\text{TGp}_{\text{peak}}$ 2.66 ± 1.06 (95%CI 2.25–3.07) and $\text{anti-Xa}_{\text{peak}}/\text{anti-Xa}_{\text{trough}}$ 7.87 ± 4.68 (95%CI 6.05–9.68). The statistically significant differences between trough and control values were observed for the same 4 tests (mean $\pm$ SD): $\text{PT}_{\text{trough}}$ 11.14 ± 0.81 vs $\text{PT}_{\text{control}}$ 10.67 ± 0.55 ; $\text{APTT}_{\text{trough}}$ 26.68 ± 2.60 vs $\text{APTT}_{\text{control}}$ 25.41 ± 2.03 ; $\text{TGp}_{\text{trough}}$ 125.02 ± 88.08 vs $\text{TGp}_{\text{control}}$ 221.02 ± 87.78 ; $\text{anti-Xa}_{\text{trough}}$ 30.84 ± 19.53 vs $\text{anti-Xa}_{\text{control}}$ 1.77 ± 3.01 .

Conclusion: Using ROC we evaluated all tests and found the anti-FXa the best – in terms of sensitivity and specificity – laboratory tool to determine rivaroxaban presence in plasma.

Disclosure of Interest: None declared.

PO050-TUE

A fully automated aid for the improvement of HIL interference management in the hemostasis laboratory: test assay-specific flagging of laboratory results for hemolysis, icterus and lipemia/turbidity

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Background: Hemolysis, Icterus and Lipemia (HIL) can interfere with laboratory tests by optical or chemical mechanisms. Manufacturers express HIL tolerance in hemoglobin (Hgb), bilirubin (Bili) and triglyceride (Tg) concentrations. Hemostasis laboratories evaluate the HIL status of a sample by visual observation that is subjective and prone to inappropriate acceptance or rejection of samples.

Aims: Instrumentation Laboratory (IL) aims to provide automated, test-specific HIL assessment with the ACL TOP Family 50 Series Hemostasis Testing Systems.

Methods: Hgb and Bili are calculated based on absorbance (abs) measured at three wavelengths. Intralipid®-derived Tg limits correspond to well-defined abs values. Abs used by the HIL feature can come from different abs source assays, PT or APTT runs, or from diluted plasma. Estimated HIL in the sample are compared to the specific HIL limits per IL test kit package inserts and the hemostasis results are flagged accordingly for HIL.

A wide variety of plasma samples were used to demonstrate matching of HIL results 1) from different abs source assays; and 2) generated by ACL TOP Family 50 Series systems vs. chemistry analyzers.

Results: HIL method comparison (MC) with different abs source assays show correlation coefficients (r) >0.98 , slopes 1.0 ± 0.06 and intercepts (y) close to zero. Testing >800 samples at three hospital laboratories with different sets of ACL TOP Family 50 Series systems, Envoy 500 (Vital) and HemoCue (HemoCue) showed combined MC results as: Hgb $r = 0.976$, slope 1.08 , $y = 12$, range $0-1500$ (mg dL⁻¹); Bili $r = 0.971$, slope 1.14 , $y = 0.7$, range $0.1-45$ (mg dL⁻¹). The individual sites showed results similar to the combined.

Conclusion: The HIL flagging capability of ACL TOP Family 50 Series systems is robust; the estimated HIL values correlate well with HIL results generated with clinical chemistry analyzers. Automated, assay-specific HIL flagging could aid furthering HIL interference management.

Disclosure of Interest: J. Polgar Employee of: Instrumentation Laboratory, H.-F. Lin Employee of: Instrumentation Laboratory, J. Havelick Employee of: Instrumentation Laboratory, M. Doyle Employee of: Instrumentation Laboratory, M. Triscott Employee of: Instrumentation Laboratory.

PO051-TUE

Handling of the Hemoclot Thrombin Inhibitor assay reagents on Sysmex CS-2100i when monitoring dabigatran in acute clinical situations

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Background: The Hemoclot Thrombin Inhibitors assay (HTI) (Hyphen biomed) has been introduced for therapeutic monitoring of patients treated with dabigatran etexilate, in specific clinical situations such as acute bleeding, thrombosis or before acute surgery. According to the supplier (Aniara) preparation time for the reagents is 1 h, when adapting the method to the Sysmex CS-2100i analyzer. This is not optimal in an acute clinical situation. Preparation time according to the reagent package insert is however only 15 min.

Aims: The objective of this study was to test the HTI reagent stability on Sysmex CS-2100i when preparing reagents according to package inserts.

Methods: Reagents, controls and calibrators were prepared according to package inserts with an incubation time of 15 min before use. Controls were measured consecutively every hour for 8 h. Additionally, freshly prepared controls were measured every hour from 3 h and onwards. The lot specific control target values were: 112 ng mL⁻¹ (C1) and 290 ng mL⁻¹ (C2).

Results: C1 values increased from 113.9 ng mL⁻¹ to 138.8 ng mL⁻¹ (22%) after 3 h, and then remained stable from 3 to 8 h (CV_{0-8 h} = 6.6%, CV_{3-8 h} = 2.9%). Likewise, C2 values increased 14%, from 281.4 ng mL⁻¹ to 321.0 ng mL⁻¹ after 2 h and then remained stable from 2 to 8 h (CV_{0-8 h} = 4.6%, CV_{2-8 h} = 1.7%). A difference of 10% (bias goal) was considered acceptable. Higher levels of differences could lead to an incorrect estimation of the patients bleeding or thrombosis risk in clinical practice, especially when values approach the borders of the therapeutic range.

Conclusion: The results of quality controls for the HTI assay varied considerably during the initial 2-3 h of use when prepared according to the package inserts. The results indicate that reagents are in need of a longer stabilization period before use, which makes the HTI assay less suitable for dabigatran monitoring in acute clinical situations.

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PO052-TUE

Time in therapeutic range and complications of antithrombotic treatment

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Background: Patients (pts) with mechanical heart valve prosthesis and atrial fibrillation are at high risk for thromboembolic complications. For them, life long anticoagulation therapy with vitamin K antagonists (VKA) is recommended.

Aims: The purpose of the study was to evaluate the possible correlation between the frequency of bleeding and thromboembolic complications and time out of therapeutic range.

Methods: Total of 384 pts divided in two groups have been included. Group of pts with atrial fibrillation consisted of 328 pts, 177 (54%) male, 151 (46%) female, age range 38-88. Group of pts with mechanical valve prosthesis included 56 pts, 27 (48%) male, 29 (52%) female, age range 26-80. Prothrombin time - INR was determined from capillary blood using thrombotest reagent and coagulometer thrombotrack, manufactured by Axis Shield, Norway. INR controls have been performed at outpatient anticoagulation clinic, Clinical Center of Vojvodina, Novi Sad.

Results: In group of pts with atrial fibrillation, total number of controls was 12684, of which INR < 2 in 4721 (37%), INR 2-4.5 in 7403 (58%) and INR > 4.6 in 560 (4%). Mean TTR was 57%. Thromboembolic complications had 29 pts and haemorrhagic complications occurred in 68 pts. In group of pts with mechanical valve prosthesis, total number of controls was 3367 of which INR < 2 in 719 (21%), INR 2-4.5 in 2434 (71%) and INR > 4.6 in 214 (6%). Mean TTR was 70%. Thromboembolic complications occurred in 3 pts and haemorrhagic complications was documented in 23 pts.

Conclusion: In group with mechanical heart valve prosthesis thromboembolic complications occurred more frequently in pts with higher percentage of controls below therapeutic range. In group with atrial fibrillation bleeding occurred more frequently in pts with higher percentage of controls over therapeutic range.

Disclosure of Interest: None declared.

PO053-TUE

Usefulness of dilute Russell's viper venom clotting time (DRVVT) for the routine monitoring of new oral anticoagulants

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Background: The new oral anticoagulants such as rivaroxaban (Bayer Healthcare) (R) apixaban (BMS/Pfizer) (A) and dabigatran (Boehringer Ingelheim) (D) have been approved to manage thrombotic and cardiovascular disorders. There have been reported bleeding

complications suggesting the need for monitoring of these agents in some patient populations.

Aims: This study was designed to determine which of the available clotting assays could be used to calculate the circulating concentration of these new oral anticoagulants in plasma.

Methods: Citrated blood was drawn from ten donors and to obtain platelet poor plasma (PPP). The PPP was supplemented with A, R and D in a concentration range of 0–1.0 $\mu\text{g mL}^{-1}$. In addition, plasma samples, containing varying concentrations of either A, R or D were also blindly analyzed. The plasma samples were analyzed using PT/INR (Innovin, Seimens, Deerfield, IL), APTT reagent (Platelin, TCoag, Ireland) dilute Russell's viper venom time (DRVVT) and DRVVT confirm (HemosIL, Instrumentation Laboratories (Bedford, MA)).

Results: In the PT and APTT assays a concentration dependent increase in clotting time was observed, however the slope of the line was not very steep. In contrast, both the DRVVT and DRVVT confirm assays the concentration-response curves were straight lines with a steep slope. Therefore, it was possible to extrapolate the concentrations of the unknown samples using both of these assays. In addition, there was a good correlation between the calculated concentration and the actual concentration in the range of 50 to 1000 ng mL^{-1} ($r^2 = 0.82$) with both assays.

Conclusion: These results suggest the dRVVT and DRVVT confirm assays are sensitive to A, D and R at concentrations $> 50 \text{ ng mL}^{-1}$. Therefore, A, D and R can be monitored by these assays. Both of these assays are commercially available and can be readily adapted to any automated instrument.

Disclosure of Interest: None declared.

PO054-TUE

Performance of Hemosil direct thrombin inhibitor (DTI) assay on the ACL top family instruments

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Background: Dabigatran (DAB), marketed as the pro-drug, Dabigatran Etxilate (Pradaxa), is an oral direct thrombin inhibitor (DTI) and an alternative to the vitamin K antagonists (VKA), warfarin or coumadin. Although fixed dosage without monitoring is indicated, information regarding plasma levels of Dabigatran would aid in making clinical decisions in situations where measurement is warranted.

Aims: Performance of the IL HemosIL Direct Thrombin Inhibitor Assay on the ACL TOP Family was evaluated to determine use of the assay to test for functional Dabigatran concentrations in citrated plasma.

Methods: A sixpoint calibration curve was prepared automatically on the ACL TOP by mixing DAB Calibrator 2 (500 ng mL^{-1}) with DAB Calibrator 1 (0 ng mL^{-1}) and fitting the points with a split math model. Linearity was assessed up to 2000 ng mL^{-1} with reflexive testing. Method comparison, precision, interference, were performed. On-board and reconstituted stabilities (2–8 °C, –20 °C) were measured for plasma components and DTI reagents.

Results: The precision was $\leq 5\%$ CV and recoveries of the DAB samples within their established limits. The test was linear between 20 – 2000 ng mL^{-1} DAB with reflexive testing. Method Comparison of R = 0.98 and slope = 1.05 was seen for IL DTI vs. commercial DAB kit. No interference was seen for hemoglobin (300 mg dL^{-1}); bilirubin (40 mg dL^{-1}); triglycerides (873 mg dL^{-1}); and heparin (2.2 IU mL^{-1}). The DTI Reagents were stable for 6 h on-board and 48 h at 2–8 °C. The plasma components were stable for 8 h on-board and 7 days at 2–8 °C. The DTI Reagents and plasma components were stable for 2 month at –20 °C.

Conclusion: The IL HemosIL DTI Dabigatran Assay on ACL TOP is reliable and accurate for measuring Dabigatran in citrated plasma. It

has good sensitivity in the low range to verify clearance of DAB prior to surgery, as well as sensitivity in the clinical and high range. The test has satisfactory component stability, good sample precision and comparison to a predicate device.

Disclosure of Interest: None declared.

PO055-TUE

In vitro correction of impaired blood coagulation in a model of severe thrombocytopenia by fibrinogen and activated prothrombin complex concentrate

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Background: Alternatives or supplements to transfusion of blood products are often needed for treatment of severe thrombocytopenia patients with bleeding.

Aims: The aim of this study was to improve clot quality and stability in reconstituted thrombocytopenia blood.

Methods: Thrombocytopenia [$(16 \pm 4) \times 10^6 \text{ mL}^{-1}$] was created by centrifugation of normal blood and mixing platelet-poor plasma with packed cells. The blood samples were subjected to clotting by CaCl_2 and tissue factor and to fibrinolysis by tPA. Blood was diluted by 40% with TRIS/saline buffer to mimic the situation when patients are subjected to massive transfusion of liquids. Clotting time (CT), α -Angle (αA), maximum clot firmness (MCF), and lysis onset time (LOT) were evaluated using thromboelastometry.

Results: Thrombocytopenia blood was spiked with fibrinogen (3.0 mg mL^{-1}) and activated prothrombin complex concentrate (FEIBA, 1 U mL^{-1}) following calibration of these agents. Spiking of non-diluted blood with FEIBA was followed by shortening of CT. αA and MCF were increased by spiking with fibrinogen and FEIBA. LOT was prolonged by FEIBA meaning inhibition of tPA-induced fibrinolysis. Further increase of αA and MCF and prolongation of LOT was observed by combining fibrinogen with FEIBA. Dilution of blood was followed by reduction of αA and MCF compared to non-diluted blood that was partly reversed by fibrinogen and FEIBA being higher using these agents in combination. Spiking of diluted blood with FEIBA together with fibrinogen led to fibrinolysis inhibition. Increasing concentration of fibrinogen (6.0 mg mL^{-1}) combined with FEIBA led to further augmentation of MCF.

Conclusion: The results of this study show that spiking of severe thrombocytopenia blood with fibrinogen in combination with FEIBA improved clot quality and stability that is especially important in thrombocytopenia complicated with hemodilution.

Disclosure of Interest: None declared.

PO056-TUE

New immunodepleted plasma for determination of Factor XI activity

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Background: Ergonomic and reliable measurement of Factor XI (FXI) activity is essential for assessing patients with FXI deficiency due to its implication in clinical management.

Aims: To assess the performance of a novel immuno-depleted FXI deficient human plasma in the one-stage FXI activity assay in various samples.

Methods: The assays were performed with the new immuno-depleted FXI deficient human plasma STA[®] – ImmunoDef XI used with STA[®] – C.K. Prest[®] (Stago) on STA-R[®]. STA[®] – Unicalibrator was used for the calibrations and Internal Quality Control (IQC) were run using STA[®] – System Control N + P. The reference method tested in parallel was STA[®] – Deficient XI used with STA[®] – C.K. Prest[®].

One hundred samples were tested in 7 runs: 26 samples from patients with a known medical history of FXI deficiency, 50 normal samples and 24 other samples (anticoagulated, lupus positive and samples spiked with FXI).

Samples were spread over the new product's working range (1–200%) and results were compared to the ones obtained with the reference method.

Correlations with and without the spiked samples were analysed using linear and Deming regressions. Differences were plotted in Bland&Altman graphs.

The results of the new product obtained with a daily calibration were compared to results obtained with the valid calibration of the first day (rerun only if IQCs were out of range).

Results: The IQC day to day reproducibility resulted in a Coefficient Variation < 3% (based on factor levels).

Results were similar when calculated with the daily calibration or the valid calibration.

The correlation between the two assays was: $y = 0.963x + 5.982$ ($r = 0.988$) (all samples).

Results were similar when removing the spiked samples from the analysis.

Conclusion: The correlation between the two FXI deficient plasma products shows equivalent results for the whole assay working range. The IQC results show good day to day reproducibility, and there is no need for a daily calibration.

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PO057-TUE

Assessment of thrombin generation test for bypassing therapy monitoring in haemophilia patient with inhibitor

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Background: The current concept of hemophilia treatment suggests using factor replacement therapy for bleeding episodes as well as for prophylactic infusions. The development of inhibitors results in decreasing of replacement therapy efficacy, so bypassing agents (BPA) are to be used. The optimal application of BPA is hampered by the lack of laboratory assays to monitor efficacy and determine adequate dosing, which is the most important in the surgical management of such patients. The thrombin generation test (TGT) may give the additional clinically relevant information.

Aims: The aim of our study was to evaluate the possibility of using TGT for the assessment of BPA efficacy in hemophilia A patient with inhibitor.

Methods: The 3-year old patient with hemophilia A and inhibitor (1.6 BU mL⁻¹) underwent a surgical procedure (venous access device was implanted). Thrombin generation was measured at Fluoroscans Ascent[®] fluorometer using TF 1pM. The following parameters were analyzed: lag time of thrombin generation (LT, min), endogenous

thrombin potential (ETP, nm*min), maximum concentration of thrombin (Peak, nm). The surgery was performed using the plasma-derived activated prothrombin complex concentrate (pd-aPCC).

Results: Before the surgery, LT was prolonged (8.5 min), and ETP and Peak were significantly reduced (131.5 nm*min and 5.3 nm, accordingly). These parameters in the control plasma were 5.6 min, 989, 4 nm*min and 98.6 nm, accordingly. Pd-aPCC 70 UE kg⁻¹ infusion led to the acceleration of thrombin generation (LT came down to 3.5 min), but ETP and Peak didn't change enough. The patient exhibited the bleeding, so he was given pd-aPCC 100 UE kg⁻¹. The bleeding was stopped and TGT parameters' correction was observed (LT was 2.6 min, ETP – 1058 nm*min, Peak – 92.2 nm).

Conclusion: TGT may be helpful for predicting the individual bleeding risk and for providing individual treatment regimens, especially in patients with inhibitors, but future research is required.

Disclosure of Interest: None declared.

PO058-TUE

Evaluation of a new assay for the measurement of the direct FXa inhibitor Rivaroxaban (DG-Chrom ANTI-Xa) on the Q Hemostasis analyzer (Grifols)

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Background: Rivaroxaban, one of the novel oral anticoagulants does not routinely require monitoring. However, in certain groups including renal patients, monitoring may be required.

Aims: Aim of the study was to evaluate the performance characteristics of a new method DG-Chrom Anti-Xa (Grifols, Spain) for quantitative assessment of the direct FXa inhibitor Rivaroxaban.

Methods: Test plasma is diluted in buffer containing a heparin inhibitor and incubated with an excess of FXa. After incubation a chromogenic substrate is added and residual FXa cleaves the substrate producing colour at 405 nm. The assay is performed on Q-analyser (Grifols, Spain). The reference Rivaroxaban assay, also utilising heparin inhibition was Biophen DiXal (Hyphen, France) performed on Sysmex CS2100 (Sysmex, UK).

Results: Calibration curves ($n = 5$) produced r values > 0.998. Between assay precision was checked at three levels of QC ($n = 5$): High (305 ng mL⁻¹) CV 3.8%, Medium (146 ng mL⁻¹) CV 3.7% and Low (64 ng mL⁻¹) CV 9.5%.

UFH group ($n = 16$, mean 0.65 IU mL⁻¹, median 0.69 IU mL⁻¹) and LMWH group ($n = 20$, mean 1.08 IU mL⁻¹, median 0.75 IU mL⁻¹) exhibited no interference, rivaroxaban estimation below assays limit of detection (LoD) of 15 ng mL⁻¹. Tinzaparin sample with an elevated LMWH heparin level of 8.6 IU mL⁻¹, generated apparent rivaroxaban level of 20.8 ng mL⁻¹, below assays Limit of Quantification (LoQ) of 40 ng mL⁻¹. Plasma spiked with UFH ($n = 10$) between 1.0 and 3.0 IU mL⁻¹ or fondaparinux ($n = 10$) between 0.5 and 1.0 IU mL⁻¹ generated levels below LoQ and LoD respectively.

In rivaroxaban group DG-Chrom Anti-Xa (mean 152 ng mL⁻¹, median 146 ng mL⁻¹) and Biophen DiXal (mean 161 ng mL⁻¹, median 146 ng mL⁻¹) correlated well ($n = 45$), $r = 0.9738$. Overall ($n = 123$), the assays gave good correlation between methods in all samples tested, $r = 0.991$.

Conclusion: DG-Chrom Anti-Xa assay compares well with the Biophen DiXal assay for rivaroxaban measurement. The heparin inhibitor contained within the buffer prevents any interference from UFH, fondaparinux and LMWH at therapeutic levels.

Disclosure of Interest: None declared.

PO059-TUE

How the direct oral anticoagulant apixaban affects thrombin generation parameters

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Background: Apixaban is a direct oral anticoagulant (DOAC) targeting factor Xa and thus quenching thrombin generation and clot formation.

Aims: To investigate the influence of apixaban on the parameters of thrombin generation.

Methods: Aliquots of a pooled normal plasma have been added with increased concentrations of purified apixaban and were used to assess the degree of modification brought about by the drug on the basic tests of coagulation prothrombin and activated partial thromboplastin time (PT and APTT) and on thrombin generation parameters.

Results: The study shows that while apixaban has little effect on PT or APTT it does affect all the parameters of thrombin generation, including the lag-time (which is increased), the endogenous thrombin potential (ETP) and thrombin-peak (both decreased although to a different extent), and the velocity index (decreased). Interestingly, the above effects were more pronounced when the measurements were recorded in the presence of thrombomodulin, thus making the ratio (with/without thrombomodulin) to decrease consistently as a function of the apixaban concentrations.

Conclusion: These findings support the antithrombotic properties of apixaban and can help to understand the mechanism(s) of action of this drug. Thrombin generation could be used as a convenient laboratory tool to assess the anticoagulant activity of other drugs and to make between-DOAC comparison.

Disclosure of Interest: None declared.

PO060-TUE

The correlation of atypical aPTT curves with type of coagulation defect

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Background: The last generation optical coagulometers (ACL Top) allow to display of clot reaction curves with superimposed first and second derivative curves. In our study, we would like to demonstrate the importance of atypical biphasic types of curves for detection of coagulation defects.

The graphical representation of the clot reaction can provide very useful information. New software in the device allows to display clot reaction curves with superimposed first and second derivative curves.

Aims: In our study, we observed an atypical curve in 44 from 74 consecutive sent samples with pathological aPTT. The curves have different biphasic course in contrary to the classic 'S' shape curves observed in normal patients.

The aim of our study was to perform correlation of aPTT pathology in differential diagnosis with atypical waveforms.

Methods: The detection of aPTT was performed on ACL Top analyzer. We used aPTT reagent with synthetic phospholipids.

In the differential diagnosis of pathological aPTT the level of vWf, FVIII, F IX, FXI and FXII, the inhibitors of coagulation factors and the LA were detected.

Results: In our group, the presence of pathological aPTT curves was in 43 samples from 74 (atypical curves in 58%). For a detailed evaluation

of the differential diagnosis aPTT factor deficiency were detected in 38 patients (89%), 4 patients had specific inhibitor, 1 patient vW factor and Fletcher factor (100%).

The opposite situation was in 19 patients with lupus anticoagulant who had pathological curves only in 15%. These patients with one exception had comorbid another defect.

Conclusion: The presence of atypical course of aPTT curve can help us in the differential diagnosis of pathological aPTT. With a relatively high probability it helps us to identify the risk of bleeding condition based on deficit factors or the presence of an inhibitor. LA on the other hand, shows the presence of atypical aPTT curves.

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Disclosure of Interest: None declared.

PO061-TUE

Efficacy of novel oral anticoagulants (NOACs) using diluted Russell's viper venom test (dRVVT)

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Background: Novel oral anticoagulants (NOACs) show a more predictable dose-dependent response than warfarin and their use does not require routine monitoring of blood coagulation. However, anticoagulation testing becomes necessary in case of poor compliance or in an emergency situation.

Aims: In this study, we determined the characteristics of extended clotting time using diluted Russell's viper venom test (dRVVT).

Methods: In this study, clotting time was measured by the dRVVT Confirm test (dRC) with high phospholipid concentration, using the ACL-TOP coagulometer. Plasma from healthy donors were collected in tubes containing citrate and an anticoagulant (dabigatran, rivaroxaban, or apixaban) was added to each sample. In addition, plasma samples were collected from patients taking these medications orally, at a random interval after taking the drug, during their visit to our institute for a medical examination. The samples from patients were assessed in terms of PT, APTT, dRC, and chromogenic substrate method.

Results: dRC result was prolonged in an NOAC dose-dependent manner, but eventually the rate of prolongation of coagulation time reached a plateau. Apixaban (200 ng mL⁻¹) added *in vitro* to blood from healthy donors prolonged the dRC by about 1.5 times, as opposed to both PT and APTT, which were prolonged by less than 1.2-fold. As compared to control samples, the dRC prolongation ratios of samples from patients taking dabigatran, rivaroxaban, apixaban, and oral warfarin were 2.12 ($n = 46$), 1.65 ($n = 51$), 1.44 ($n = 21$), and 1.39 ($n = 21$), respectively. Although dRC prolongation in lupus anticoagulant (LA)-positive cases ($n = 7$) was barely prolonged, by approximately 1.1-fold, that of LA-positive patients taking warfarin ($n = 7$) was prolonged 1.34-fold.

Conclusion: dRC was found useful for detecting overdose of anticoagulation by NOACs, since not only was a clear prolongation of coagulation time obtained, but its results were relatively less affected in LA-positive samples.

Disclosure of Interest: None declared.

PO062-TUE

Ex-vivo and in-vitro comparative assessment of MOD-5014 (Factor VIIA-CTP), a novel long-acting coagulation factor to recombinant FVIIA

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Background: OPKO(f.k.a PROLOR) is a clinical stage company developing long acting versions of therapeutic proteins utilizing CTP technology. This involves fusion of the hCG C terminus peptide to proteins. The technology was clinically validated for several drugs while maintaining their biological activity

Aims: To compare the *ex-vivo* and *in-vitro* activity of MOD-5014 to rhFVIIa as part of the preparation for First in Human Study in hemophilic patients and to provide a solid dose selection rational

Methods: MOD-5014 was expressed in CHO cells, purified and activated utilizing a CTPspecific purification process. MOD-5014 activity was compared to commercial rhFVIIa in a dose-dependent manner by: STA-Clot, Factor X activation and FVII chromogenic assays and by comparing the affinity to TF by SPR. In addition, the thrombin generation and ROTEM performance of MOD-5014 in hemophilic patients plasma was characterized at a range of concentrations by either recalcification of samples and/or activation of the extrinsic or intrinsic pathway while rh FVIIa served as comparator. Finally, PT and aPTT were assessed.

Results: MOD-5014 and rhFVIIa demonstrated a comparable *in-vitro* activity as reflected by equivalent clotting time and EC50. In addition, MOD-5014 affinity to TF, as assessed by SPR, was similar to rhFVIIa. Comparison of the ability at wide range of MOD-5014 and rhFVIIa concentrations to induce TG showed proper activation as reflected by the lag time, peak height, and ETP. Preliminary assessment of MOD-5014 in ROTEM system suggests similar dose-dependent effect of both agents

Conclusion: Our data suggest that attachment of CTP to FVIIa maintains comparable functionality and affinity as those of rhFVIIa. These results provide a solid rational for the proposed dose range to be used in the upcoming MOD-5014 clinical study in hemophilic patients and implies that MOD-5014 has the potential of being a promising and effective long acting rhFVIIa.

Disclosure of Interest: None declared.

PO063-TUE

Impact of edoxaban on haemostasis diagnosis assays: practical recommendations

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Background: Edoxaban may affect the results of coagulation tests routinely used in case of thrombophilia or in exploration of a haemorrhagic event.

Aims: To provide laboratory recommendations for the accurate interpretation of haemostasis laboratory assays that may be influenced in patients treated with edoxaban.

Methods: Edoxaban was spiked at increasing concentration (0 to 500 ng mL⁻¹) in normal citrated human platelet-poor plasma to assess Thrombin Time (TT), Ecarin Clotting Time (ECT), Reptilase Time (RT), testing for lupus anticoagulant (dilute Russell Viper Venom Time (dRVVT), PTT-LA[®] and StacLOT-LA[®]), Activated Protein C Resistance (APC-R), measurement of clotting factors (XII; XI; IX;

VIII; VII; V; X and II), antithrombin measurement (with thrombin and factor-Xa based assay), fibrinogen measurement (Clauss and PT-derived method) and free Protein-S measurement (clotting method).

Results: TT, ECT, RT, fibrinogen (Clauss) and the APC-R were not influenced by edoxaban. dRVVT, PTT-LA[®] and the StacLOT-LA[®] were both prolonged in a concentration dependent manner in presence of therapeutic concentrations (i.e. around 150 ng mL⁻¹ at C_{max}) of edoxaban. PT and aPTT-based clotting factor measurements were also highly influenced at these concentrations. Only FII seemed to be less affected. However, it depends on the sensitivity of the PT and aPTT reagents. High edoxaban concentrations (i.e. 500 ng mL⁻¹) interfered with the measurement of free protein-S (increase of 22% at 500 ng mL⁻¹ of edoxaban) and antithrombin, using factor Xa-based chromogenic assay (15% at 500 ng mL⁻¹), and with the measurement of PT-derived fibrinogen.

Conclusion: Edoxaban mainly interferes with chromometric or chromogenic assays involving FXa or upstream coagulation factors. When these tests are used, it is mandatory to take into account the inter-reagent variability in terms of sensitivity as well as the delay between the last intake of the drug and the blood sampling.

Disclosure of Interest: None declared.

PO064-TUE

Introduction of a liquid anti-Xa method for quantification of current therapeutic Xa inhibitors

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Background: Assessment of anti-Xa activity by chromogenic methodology is well established in diagnostic laboratories for patients receiving unfractionated (UFH) or low molecular weight heparin (LMWH). Reconstitution of lyophilised reagents and generation of calibration curves for each run directly from prescription product, is complex and time consuming. Availability of a wide range of Xa inhibitors and reduction in routine monitoring assay is now predominantly required urgently in bleeding or thrombotic complications. Availability of liquid based reagents in conjunction with commercial calibrators potentially provides analytical tools to support a responsive diagnostic service.

Aims: Using liquid anti-Xa reagent and commercial calibrators to assess quality assurance of application of stored calibration curves and within and between batch quality control (QC) for quantifying the anti-Xa activity in plasma of UFH, LMWH, Danaproid, Rivaroxaban and Apixaban.

Methods: Assays performed on Destiny Max analyser (Stago UK). Anti-Xa activity was measured using Liquid Anti-Xa reagent and Multi Hep Calibrator for UFH and LMWH, STA Rivaroxaban and Apixaban calibrators and associated QC plasmas (Stago UK). Danaproid calibrators and QC plasmas (Technoclone Ltd).

Results: LMWH within batches CV% = 8.2, 6.8, 4.7 (low QC) and 6.8, 6.2, 8.4 (high QC) respectively and between batches CV% = 8.5 (low QC) and 10.8 (high QC). UFH between batches CV% = 8.04 (low QC) and 7.53 (high QC). Rivaroxaban within batches CV% = 2.88, 8.19, 10.2 (low QC) and CV% = 3.98, 4.25, 7.86 (high QC) respectively and between batch CV% = 9.75 (low QC) and 6.04 (high QC). Apixaban between batches CV% = 6.86 (low QC) and 5.29 (high QC) and Danaproid CV% = 2.08 (low QC) and 0.83 (high QC).

Conclusion: Xa inhibition for all anticoagulants showed good within and between batch reproducibility and quality assurance using stored calibration curves for liquid anti-Xa reagent, providing a robust assay suitable for application for patients in clinical emergencies.

Disclosure of Interest: None declared.

PO065-TUE

Preliminary performance data of a new chromogenic direct anti-Xa assay evaluated for the quantification of Rivaroxaban

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Background: Rivaroxaban is an oral direct factor Xa inhibitor approved for a series of thromboembolic indications. Routine monitoring is not required. However, measurement of plasma drug levels may become beneficial in certain clinical situations.

Aims: To develop a new automated assay for the quantification of direct anti-factor Xa inhibitors such as Rivaroxaban in human plasma.

Methods: A one-stage, competitive, chromogenic assay*, a Rivaroxaban standard* and Rivaroxaban quality controls* were developed. Diluted samples are mixed with a reagent containing an excess of factor Xa and with a factor Xa-specific chromogenic substrate. In the presence of Rivaroxaban, the velocity of chromogenic substrate cleavage by factor Xa is reduced which is quantified by absorbance at 405 nm.

Results: Precision, linearity of the measuring range, accuracy of results and interference by heparin were assessed with automated applications on BCS® XP and the Sysmex® CS-2100i systems. The assay's analytical measuring range expands from 0 to 350 ng mL⁻¹, and reaches a linear clinical reportable range of 20 to 1050 ng mL⁻¹ by automated sample dilution. The total precision CV of the application on CS-2100i was 2.3% for a sample with 65 ng mL⁻¹ and 6.3% for a sample with 29 ng mL⁻¹. Rivaroxaban values correlate strongly to those obtained by liquid chromatography tandem mass spectrometry ($r = 0.995$ on BCS XP, $r = 0.996$ on CS-2100i). No interference against unfractionated or low molecular weight heparins up to a concentration of 10 IU mL⁻¹ has been detected.

Conclusion: The new chromogenic direct anti-Xa assay in combination with a dedicated Rivaroxaban Standard and Rivaroxaban Controls is suitable for the automated routine quantitation of Rivaroxaban in human citrated plasma samples. (*) Products under development. Not available for sale.

Disclosure of Interest: S. Pilgrim Employee of: Siemens Healthcare Diagnostics Products GmbH, V. Esch Employee of: Siemens Healthcare Diagnostics Products GmbH, K. Kolbe-Scheu Employee of: Siemens Healthcare Diagnostics Products GmbH, B. Krumpholz Employee of: Siemens Healthcare Diagnostics Products GmbH, M. Schleifer Employee of: Siemens Healthcare Diagnostics Products GmbH, A. Lichte Employee of: Siemens Healthcare Diagnostics Products GmbH.

PO066-TUE

Preliminary evaluation of a new chromogenic assay for the quantitative determination of dabigatran in human plasma

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Background: Dabigatran etexilate is an oral direct thrombin inhibitor (DTI) used for the prevention of venous thromboembolism after elective hip and knee replacement, the prevention of stroke during arterial fibrillation, and the treatment of acute deep vein thrombosis. The active metabolite, dabigatran, has a predictable pharmacokinetic profile with no need for routine monitoring. Nevertheless, in certain clinical situations it may be beneficial to quantify the dabigatran concentration in patient specimens.

Aims: The development of an automated chromogenic assay for the quantitative detection of dabigatran in human plasma.

Methods: A thrombin-dependent assay* using a chromogenic measuring principle and respective dabigatran standards* and controls* were developed. The measurement is performed by adding an excess of thrombin and a chromogenic substrate to a citrated plasma sample. Substrate cleavage is determined by the increase in absorbance at 405 nm. The release of dye is inversely proportional to the inhibitory activity of dabigatran in the plasma sample, i.e., the smaller the dabigatran concentration, the higher is the absorbance signal per time unit. Applications were developed for Siemens BCS® XP System and Sysmex® CS-2100i System.

Results: The new DTI assay is calibrated in a range of 0–500 ng mL⁻¹ dabigatran. A clinical reportable range (CRR) of 25–1000 ng mL⁻¹ is achieved by sample predilution. On both systems, linearity was shown over the whole CRR. A total precision CV of 2.6 ng mL⁻¹ (CS-2100i) and 3.1 ng mL⁻¹ (BCS XP) was shown for a sample with 35 ng mL⁻¹ and 2.0% (CS-2100i) and 3.1% (BCS XP) for a sample with 404 ng mL⁻¹ dabigatran. Method comparison to liquid chromatography tandem mass spectrometry revealed a correlation coefficient of $r = 0.991$ (BCS XP).

Conclusion: The new DTI assay offers together with dabigatran standards and controls a reliable method for the determination of dabigatran in human plasma.

(*) Product under development. Not available for sale.

Disclosure of Interest: J. Mueller Employee of: Siemens Healthcare Diagnostics Products GmbH, S. Halilovic Employee of: Siemens Healthcare Diagnostics Products GmbH, I. Kieper-Rupp Employee of: Siemens Healthcare Diagnostics Products GmbH, D. Koch Employee of: Siemens Healthcare Diagnostics Products GmbH, J. Kurz Employee of: Siemens Healthcare Diagnostics Products GmbH, A. Lichte Employee of: Siemens Healthcare Diagnostics Products GmbH.

PO067-TUE

Prediction of bleeding tendency in hemophilia A and B using thrombodynamics assay

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Background: Bleeding tendency in hemophilia usually correlates with residual factor level, but, in a considerable number of cases, factor level cannot predict its clinical severity. Thrombodynamics is a novel integral assay of hemostasis that evaluates propagation of fibrin clot from the activating surface. Coagulation is activated by immobilized tissue factor and propagates in thin layer of non-stirred plasma. Fibrin clot formation is monitored by light scattering and spatial rate of clot growth is measured.

Aims: The aim of this study was to evaluate ability of thrombodynamics to predict bleeding tendency in hemophilia A and B.

Methods: 81 adult patients with various clinical severity of hemophilia were recruited for this study after obtaining an informed consent. For 29 hemophilia A patients and for 18 hemophilia B patients who had a background factor level bleeding phenotype was evaluated. 43 apparently healthy individuals formed a control group.

Results: Addition of phospholipid vesicles to plasma was necessary to provide sufficient sensitivity of the test to factors VIII and XI. Statistically significant correlation between factor level in plasma and rate of

clot growth was found: $r = 0.85$ for factor VIII and 0.76 for factor IX, $P < 0.01$. Rate of clot growth differed significantly between control group, mild, moderate and severe hemophilia A and B patients as defined according to factor level at the moment of blood collection. Rate of clot growth discriminated between severe and non-severe bleeding phenotype with 96% sensitivity and 76% specificity. *In-vitro* spiking of plasma from severe hemophilia A patients with factor VIII concentrate revealed significant difference between individuals despite the same factor concentration.

Conclusion: These results show that thrombodynamics is sensitive to factor VIII and IX level, can reflect individual response to factor replacement, correlates with clinical manifestation of hemophilia and therefore has potential clinical utility in this field.

Disclosure of Interest: N. Dashkevich Employee of: HemaCore Labs LLC, M. Pantelev Employee of: HemaCore Labs LLC, F. Ataullakhanov Shareholder of: HemaCore LLC, Employee of: HemaCore Labs LLC, C. Negrier: None declared.

PO068-TUE

Do PT and APTT sensitivities to factors' deficiencies calculated by the H47-A2 2008 CLSI guideline reflect the deficiencies found in plasma from patients?

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Background: PT and APTT sensitivity to detect mild isolated factor deficiencies greatly varied by using different reagents and coagulometers. H47A2 guideline proposed a method to determine the sensitivity of reagent-instrument combination, but some inconsistency has been reported.

Aims: To calculate factor deficiency sensitivity by using CLSI guideline and to compare it with that obtained by analyzing data from patients with isolated factor deficiencies.

Methods: To calculate sensitivity according to H47A2 guideline different mixtures of a commercial normal pooled plasma (Normal Control Assayed, IL) and deficient plasmas on each factor were prepared ($< 1\%$ to 100% of activity). PT (PT HS+ and Recombiplastin 2G) and APTT (APTT SP, IL) were performed on mixtures in an ACL TOP 700 coagulometer. PT (% of activity) and APTT (sec) were plotted against factor level and a no linear regression equation was obtained. Sensitivity definition: factor concentration generating PT or APTT out of the reference range. PT, APTT and factor dosage from patients with isolated deficiencies were analyzed in the same way: 15 FV, 36 FVII, 19 FX, 21 Haemophilia A, 17 von Willebrand diseases, 15 Haemophilia B, 15 FXI y 24 FXII.

Results: PT sensitivity PTHS + (rabbit brain): FV 38 and 59, FVII 35 and 58, FX 56 and 64UI dL⁻¹ from guidelines and patients plasmas, respectively.

PT sensitivity Recombiplastin 2G (human recombinant): FV 39 and 45, FVII 51 and 50, FX 33 and 61 UI dL⁻¹, from guidelines and patients plasmas, respectively.

APTT sensitivities: FV 39 and 45, FX 32 and 38, FVIII 47 and 60, FIX 35 and 44, FXI 33 and 43, FXII 37 and 46 UI dL⁻¹, from guidelines and patients plasmas, respectively.

Conclusion: Reagent-coagulometer combinations evaluated have adequate sensitivity to factor deficiencies. Sensitivity calculated by H47 A2 procedure could be considered an approach and the minimum one for the reagents-instrument combination, because sensitivities obtained by analyzing data from patients plasmas were equal or higher for all factors evaluated.

Disclosure of Interest: None declared.

PO069-TUE

Markers of hemostasis activation after early discontinuation of fondaparinux in low-risk patients hospitalized with non-ST-elevation acute coronary syndrome

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Background: Discontinuation of heparin in aspirin-treated patients (pts) with non-ST-segment elevation acute coronary syndrome (NSTEMI-ACS) may be associated with reactivation of coagulation. However is not clear whether this phenomenon exists in low-risk NSTEMI-ACS pts and after short-term fondaparinux (F) use.

Aims: To assess markers of hemostasis activation after very early discontinuation of F in non-invasive treatment of low-risk pts hospitalized with NSTEMI-ACS.

Methods: 53 pts admitted with NSTEMI-ACS at median 2.3 h after last episode of chest pain were included into prospective study. All pts had GRACE score ≤ 108 , negative Tn T (cut-off 0.03 ng mL^{-1}), and no ST-segment deviation $> 0.1 \text{ mV}$. Aspirin and beta-blockers were used in all cases while clopidogrel in 35 (66%). After single subcutaneous injection of F at presentation no anticoagulants were used. Plasma thrombin-antithrombin (TAT), D-dimer (DD), plasmin-antiplasmin (PAP) levels, plasminogen activator inhibitor-1 (PAI-1) activity and its complex with tissue plasminogen activator (tPA/PAI-1) level were measured at median 18.0 and 42.0 h after F. Pts were followed until hospital discharge (median 14 days).

Results: After discontinuation of F plasma TAT, DD and PAP levels increased while PAI-1 activity and tPA/PAI-1 complex level did not significantly change (table). During hospital stay no patient died or had myocardial infarction and 3 had recurrent angina.

Markers of hemostasis activation during and after short-term F use in pts hospitalized with NSTEMI-ACS.

	On F	After F	P
TAT (ng mL ⁻¹)	3.1 (2.4–3.9)	3.3 (2.6–4.4)	0.002
DD (ng mL ⁻¹)	359 (253–555)	486 (280–952)	0.002
PAP (ng mL ⁻¹)	471 (395–577)	498 (392–656)	0.052
tPA/PAI-1 (ng mL ⁻¹)	11.8 (6.9–27.9)	12.1 (4.9–26.3)	0.64
PAI-1 (U mL ⁻¹)	15.7 (11.0–19.3)	14.7 (12.3–20.0)	0.66

Conclusion: In non-invasive treatment of low-risk pts hospitalized with NSTEMI-ACS, very early discontinuation of F was associated with activation of coagulation. However, clinical events rate was low.

Disclosure of Interest: None declared.

PO070-TUE

New insights for factor analysis with aptt and pt for Factors IX, VIII, XI and Factor V

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Background: Guidelines prescribe factor analysis by coagulation tests to be calibrated with mixtures of deficient and normal plasma. The deficient plasma should have all other coagulation factors at least at 50% and mixing has the studied factor as single limiting factor.

Recently, studies showed that factor XIa activates factor X directly, contact activation bypasses factor XI, and factor V is only partially activated.

Aims: To evaluate the consequences for factor analysis.

Methods: Standard APTT, PT methods.

Results: For factor VIII- and IX-factor analysis the activity of the bypass of factor XIa on factor X is strongly dependent of the factor XI level in the test. It requires dilutions with a constant concentration of factor XI. Magnitude of effects are dependent on APTT reagent (silica/ellagic acid). Dilution according to current guidelines compared to dilutions with constant factor XI show a difference up to a factor 2 for the low range of VIII or IX.

For factor XI, a transient clotting activity is observed during activation. This extra contact-derived activity (bypassing factor XI) is variable within a pre-incubation time of 0 – 12 min. Calibration lines show strong dependence in slope on pre-incubation time. Extra activity has largely been inactivated with a prolonged incubation time of ~10 min, which is the new preferred protocol. This factor XI bypassing activity has been the basis for differences in slope of calibration lines using 4 min incubation time for different deficient plasmas with the standard APTT.

For factor V analysis with the PT, it was observed that activation of factor V is partial and different for different levels of factor V. This violates the rule of one critical factor in the test. Furthermore, the degree of activation of factor V was different for different PT reagents.

Conclusion: It is concluded that in order to full fill the criteria of current guidelines, modifications of tests and reagents are required. In some situations the guidelines themselves require modification.

Disclosure of Interest: None declared.

PO071-TUE

Reliability of a new point-of-care portable coagulometer for PT-INR test performed in the hospital anticoagulation clinic

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Background: Recently, a new coagulation point-of-care testing (POCT) system, the microINR portable coagulometer (Instrumentation laboratory-IL, Italy), has been introduced for assessing PT-INR in capillary whole blood samples of patients on oral vitamin K antagonist (VKA) therapy.

Aims: This study was aimed to evaluate the precision and accuracy of this POCT coagulometer.

Methods: Capillary whole blood PT-INR by using POCT device were assessed in 288 patients on oral VKA therapy. At the same time, citrated blood was withdrawn for comparing citrated plasma PT-INR results obtained from a laboratory conventional method (ACL TOP700, IL, Italy).

Results: Significant correlations were observed between POCT and laboratory PT-INR ($\rho = 0.96$, $P < 0.001$, $n = 288$). For all determinations, the agreement analysis by using Bland Altman plot revealed that the mean differences between POCT system and laboratory method was: 0.05 ± 0.4 INR ($P < 0.01$). The 95° limits of agreement ranged from -0.7 to 0.85 INR ($P < 0.001$). Across different POCT PT-INR ranges, the following mean differences were observed: < 2.0 INR ($n = 20$), 0.03 ± 0.24 INR; 2 to 3 INR ($n = 83$), 0.10 ± 0.29 INR; 3 to 4 INR ($n = 83$), 0.09 ± 0.33 INR; > 4 INR ($n = 102$), -0.04 ± 0.44 INR. In addition, for these different ranges significant correlations between POCT and laboratory PT-INR were observed (at least, $P < 0.01$). Regarding between-cartridge imprecision analysis, the coefficient of variation (CV) within day was: $CV = 3.2\%$. The Integrated Quality Control for the guaranteed reliability of system did not failed.

Conclusion: These data show that the POCT system microINR provides precise and accurate results that are significantly comparable with plasma laboratory PT-INR.

Disclosure of Interest: None declared.

PO072-TUE

Strenuous exercise induces a prothrombotic state that is more pronounced in men than in women

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Background: Exercise is healthy and should be recommended. Strenuous exercise, however, enhances the risk of vascular thrombotic events and increases the incidence of primary cardiac arrest.

Aims: To study the effects of strenuous exercise on risk factors of cardiovascular disease.

Methods: 93 healthy volunteers who participated in a cycling tour were tested before and directly after the race. Coagulation was tested by measuring thrombin generation in platelet poor plasma, platelet rich plasma and in whole blood and by measuring the levels of FVII, active FVII, FVIII, antithrombin, Protein C and S. Platelet reactivity in whole blood was tested by quantifying P-selectin expression and binding of fibrinogen to activated $\alpha_{IIb}\beta_3$ after platelet activation by GPVI, PAR-1, PAR-4, P2Y₁₂ agonists. The levels of fibrinogen and D-dimer were determined to study the effect on fibrin formation and the levels of VWF and active VWF to verify endothelium activation. The effect of exercise on the immune system was determined by measuring the concentration of 27 cytokines. In addition, the level of troponin T was determined.

Results: Strenuous exercise has a pro-coagulant effect. FVIII and active FVII increased while FVII zymogen, protein C and S decreased. Considering thrombin generation, a shortening of the lag time and time to peak and an increased velocity index was detected. The decreased fibrinogen and increased D-dimer levels indicated fibrin formation. Platelet reactivity increased; an effect that was more pronounced in men than in women. Exercise activated the endothelium and the immune system as indicated by elevated levels of VWF and active VWF, and elevated concentrations of IL-6, IL-8, MCP-1, RANTES and PDGF, respectively. Finally, after strenuous exercise the level of troponin T was severely increased.

Conclusion: Strenuous exercise triggers a hypercoagulable state, platelet hyperreactivity, activation of the endothelium and the immune system, and troponin T release.

Disclosure of Interest: None declared.

PO073-TUE

Whole blood platelet activity measurements multiplexed with quantitative fibrinogen, clot time and fibrinolysis measurements by T2MR

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Background: There is a clinical need for a global hemostasis diagnostic that can identify coagulable conditions and correlate with laboratory gold standards for clot time, fibrinogen, platelet activity, and fibrinolysis measurements. T2 magnetic resonance (T2MR) technology enables monitoring of the physical states of blood during coagulation for determination of these factors.

Aims: Here we expand T2MR hemostasis to a multiplexed panel and evaluate its performance for measuring clot time, fibrinogen, platelet function and fibrinolysis compared to gold standard laboratory methods.

Methods: 20 μ L samples of citrated whole blood were activated by (i) tissue factor (TF) or (ii) ellagic acid (EA) reagents. A range of clot times (CT) were produced by spiking in Rivaroxaban. A range of

fibrinogen levels were produced using blood cells that were reconstituted with fibrinogen-depleted plasma spiked with fibrinogen. Platelet activity studies utilized titrations of the platelet inhibitor ReoPro. Fibrinolysis was induced by addition of varying amounts of tissue plasminogen activator (tPA). Reference values were obtained using Stago ST4, Chrono-Log and Thromboelastography.

Results: Activated blood samples produced T2MR signals that correlated to each diagnostic result from the respective comparative method. T2MR was able to detect a range of CTs with both TF and EA reagents with a precision of < 5% and a correlation to plasma CT of $R^2 > 0.94$. T2MR detected 100–600 mg dL⁻¹ fibrinogen with a correlation to Clauss measured plasma fibrinogen of $R^2 > 0.95$. T2MR reported platelet activity that titrated with the platelet inhibitor ReoPro. T2MR detected clot contraction and subsequent clot lysis with the addition of tPA to the sample.

Conclusion: These studies show that T2MR detects a range of clinically valuable parameters associated with the hemostatic condition of a blood sample that correlate with gold-standard methods. Providing these measurements could impact patient management by providing rapid, comprehensive coagulation data.

Disclosure of Interest: None declared.

PO074-TUE

Variability in normal ranges of thrombin generation between laboratories is wide and dependent on test methodology

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Background: Thrombin generation (TG) assay is a method of assessing global hemostatic function. There is currently limited data on normal ranges of TG.

Aims: This study aims to determine normal ranges for TG using Calibrated Automated Thrombogram (CAT, Stago, France) and compare results to data from other studies.

Methods: Methods used are in accordance with manufacturer recommendations. Blood samples were collected and double centrifuged at room temperature for 5 min at 2000 g, followed by 10 min at 9400 g to obtain platelet-poor-plasma (PPP). PPP is quick-frozen and stored at -80°C until tested for TG. TG is analysed using CAT to generate lag time (LT), peak thrombin generation, time to peak thrombin generation (tpeak) and endogenous thrombin potential (ETP). Data is analysed using ThrombinoscopeTM software (Thrombinoscope BV, Maastricht, the Netherlands).

Results: 74 healthy subjects (85% ethnic Chinese, 5% Malays, 4% Indians, 6% others) participated. The median values and 5th to 95th percentile ranges for LT, peak thrombin generation, tpeak and ETP in the subjects were 2.500 (1.720–3.245) min, 277.60 (223.53–353.63) nmol L⁻¹, 5.00 (4.13–6.50) min and 1323 (1066–1676) nmol L⁻¹ per min. This differs substantially from a Chinese study whose results ranges were 2.465–4.825 min, 291.92–441.59 nmol L⁻¹, 4.341–8.443 min and 1794–2840 nmol L⁻¹ per min respectively. A Caucasian study reported similar ranges of 2.47–4.97 min, 325.6–498.5 nmol L⁻¹, 4.43–7.53 min and 1475–2368 nmol L⁻¹ per min respectively. Different methods were used in all 3 studies. The Chinese study used a double centrifugation at 2500 g for 15 min to obtain PPP while the Caucasian study used a double centrifugation at 2000 g for 10 min.

Conclusion: There is marked variability in normal ranges established by laboratories using different methodologies and test subjects, highlighting the need for a common standard and importance of individual laboratories establishing their own normal TG ranges.

Disclosure of Interest: None declared.

PO075-TUE

Resistance to thrombomodulin but not to activated protein C in cirrhotic patients

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Background: Cirrhotic patients have an impairment of haemostasis and are exposed to both thrombotic/bleeding events. Thrombin generation assay (TGA) could be informative to evaluate the coagulation step. Studies using thrombomodulin (TM) demonstrated a 'resistance' to TM. However, different mechanisms such as PC deficiency may be involved in this 'resistance'. The use of activated protein C (aPC) can by-pass the PC deficiency.

Aims: The aim was to compare the TGA with TM or aPC in cirrhotic patients and controls.

Methods: Patients with confirmed cirrhosis (free of hepatocellular carcinoma, thromboembolic event, familial history of thromboembolism or thrombophilia and not anticoagulated) were prospectively included. Blood sampling was realized at least two month after an infectious or inflammatory complication. TGA (triplicate) were performed in the presence/absence of TM (4 nM) or aPC (1 nM). Coagulation was initiated by 5 pM of tissue factor with 4 μM of phospholipids. Results were expressed as ratios with/without TM or aPC, after normalization by a standard plasma.

Results: Thirty seven Child A, 18 B, 4 C and 26 controls were enrolled. Comparatively to controls, PC and protein S levels were decreased and FVIII level and FVIII/PC ratio were increased in cirrhotic patients. ETP ratios with TM were statistically different between controls vs. Child A ($P < 0.01$), controls vs. Child B ($P < 0.001$) and controls vs. Child C ($P < 0.01$). Peak height ratio with TM were statistically different between controls vs. Child A ($P < 0.01$), controls vs. Child B ($P < 0.001$) and controls vs. Child C ($P < 0.01$). All these significant differences disappear with aPC, suggesting that the 'resistance' to the TM is due, at least partially, to the acquired deficiency in PC.

Conclusion: The main finding of this study is that PC deficiency actively participates to the TM 'resistance' in cirrhotic patients. Further studies are needed to evaluate the involvement of the FVIII increase in this resistance and the association between TGA and thrombotic events.

Disclosure of Interest: None declared.

PO076-TUE

Hypercoagulability and high residual platelet reactivity in ACS patients on dual antiplatelet therapy

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Background: It is recognized that patients with acute coronary syndrome (ACS) on treatment with dual antiplatelet therapy (aspirin + clopidogrel or prasugrel) might present a high residual platelet reactivity (RPR). As platelet activation and clotting activation influence each other, the occurrence of hypercoagulability in ACS patients might play a role in the low responsiveness to these antiplatelet agents.

Aims: This study was aimed to investigate the presence or not of a state of hypercoagulability in ACS patients undergone Percutaneous Coronary Intervention (PCI) on dual antiplatelet therapy by using thrombin generation test and thromboelastographic analysis.

Methods: Citrated whole blood was taken from 72 patients 48–72 h after PCI. Light transmission platelet aggregometry (LTA) in platelet-rich plasma induced by 10 μmol L⁻¹ ADP and 1 mmol L⁻¹ arachi-

donic acid (AA), thrombin generation test by Calibrated Automated Thrombogram (CAT, Stago, France) in platelet-poor plasma and rotational thromboelastography analysis (thromboelastometry) by using ROTEM device (TEM Int, Germany) in whole blood were performed. RPR was defined in the presence of LTA $\geq 70\%$ (ADP stimulus) and $\geq 20\%$ (AA stimulus).

Results: Patients showing RPR were 32/72. For CAT assessment, the parameters of thrombin generation were significantly higher and faster respect to those of patients without RPR: ETP,+16%; Peak,+24%; Velindex,+38%; TTPeak-11% (at least, $P < 0.01$). For ROTEM analysis enhanced fibrin formation (CFT,+11%, MaxVel,+18%, MaxVelT,-5% and AUC,+5%) and a high global elasticity and dynamic formation of clot (G,+20%; TPI,+44%) were observed (at least, $P < 0.01$).

Conclusion: These results suggest that in ACS patients a state of blood clotting activation might be related to high RPR. Hence, alternative approaches might be useful to optimize different therapies in ACS patients.

Disclosure of Interest: None declared.

PO077-TUE

Emergency assesment of patients in DOACs: the role of point-of-care testing

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Background: Despite not necessitating routine monitoring, the measurement of direct oral anticoagulants (DOACs) activity is of imperative importance in emergency conditions. Specific laboratory tests are emerging but they are time consuming and not readily available. Point-of-Care Tests (POCT) may bridge this gap and be used as a bedside solution.

Aims: To this end, we evaluated the feasibility of POCT in correlating Activated Coagulation Time (ACT) and INR with plasma levels Dabigatran and Rivaroxaban, to identify subjects at low bleeding risk (Dabigatran $< 50 \text{ ng mL}^{-1}$ and Rivaroxaban $< 60 \text{ ng mL}^{-1}$) and those at high bleeding risk (> 200 or $> 250 \text{ ng mL}^{-1}$, respectively).

Methods: ACT and INR (ACT-LR Cremascoli&Iris, Milan, Italy; INR Coaguchek XS pro, Roche Diagnostics, Monza, Italy) were measured in 69 consecutive patients with atrial fibrillation: 42 treated with dabigatran and 27 with rivaroxaban. Measurements were performed at peak (2 h after drug administration) and at trough (12 and 24 h after last dose). The results were compared with the specific tests 'diluted Thrombin Time' (Dabigatran) and 'anti-Xa activity' (Rivaroxaban).

Results: We noted a good test-drug level correlation. Correlation coefficients were 0.84 for ACT and 0.76 for INR (dabigatran 84 and rivaroxaban 54 determinations). Cut off values with the best performances to identify low and high bleeding risk patients according to the pre set drug concentrations, were 175 and 220 s for ACT (Dabigatran) and 1.15 and 1.35 INR (for Rivaroxaban). None of the patients treated with dabigatran, irrespective of the drug concentration, had levels of ACT < 150 s, considered the maximum reference level in the absence of anticoagulants.

Conclusion: In emergency situations, when other tests are not readily or timely available, ACT and INR using POCT give useful bedside information about the anticoagulation status of the patient.

Disclosure of Interest: None declared.

PO078-TUE

Evaluating dabigatran and its specific reversal with idarucizumab using a fully automated thrombelastograph TEG[®] 6s

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Background: New oral anticoagulants (OAC) do not require routine monitoring however measuring drug levels may be needed in clinical situations such as trauma and emergent surgery. Clinical or laboratory whole blood point of care assays are not established for dabigatran. Thrombelastography (TEG[®]) has shown promising results in detecting dabigatran through changes in hemostatic parameters.

Aims: This study evaluated the effect of dabigatran and its specific reversal agent with the next generation fully automated TEG[®] 6S system.

Methods: The TEG[®] 6S system (Haemonetics Corp., Braintree, MA) is based on viscoelasticity measurements using resonance frequency and disposable multi-channel microfluidic cartridges. Blood and plasma from healthy volunteers were spiked with increasing concentrations of dabigatran and tested with an OAC cartridge with an ecarin-based, direct thrombin inhibitor (DTI) channel. This channel was also evaluated with porcine plasma from an experimental trauma model ($n = 6$), measuring dabigatran pre and post trauma and after dosing with idarucizumab. Dabigatran levels were measured with dilute TT (Hemoclott[®]). TEG[®] 6s R-time (reaction time) was correlated to the drug levels.

Results: *In vitro*: R-time was highly correlated with dabigatran levels in whole blood ($r^2 > 0.95$). Significant correlation was also observed in plasma ($r^2 > 0.6$).

Trauma model: R-time was significantly correlated with dabigatran plasma levels ($r^2 = 0.7$). R-time at baseline was $(3.4 \pm 0.6 \text{ min}, n = 4)$. Following dabigatran administration, significant elongation of R-time was observed pre and post trauma with R-times $\geq 21 \pm 5.9 \text{ min}$. Administration of idarucizumab returned R-time to baseline ($3.6 \pm 0.6 \text{ min}$).

Conclusion: TEG[®] 6s has the potential to measure the effect of dabigatran on the hemostasis system effectively in whole blood as well as plasma in a clinical setting. This novel technology is fully automated and can provide clinically relevant results with whole blood in as little as 5 min.

Disclosure of Interest: F. Zaman Employee of: Haemonetics Corporation, M. Doubleday Employee of: Haemonetics Corporation, O. Grottke Grant/Research Support from: Novo Nordisk, Biotest, CSL Behring, Boehringer Ingelheim, Nycomed, Consultant for: Bayer healthcare, Boehringer Ingelheim, CSL Behring, Portola, J. Van Ryn Employee of: Boehringer Ingelheim Pharma GmbH & Co.

PO079-TUE

Effect of the residual platelet count on different coagulation parameters in freeze-thaw plasma samples at -20°C

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Background: Coagulation testing needs precise centrifugation conditions. CLSI recommends 1500 g as gold standard. Many centrifuges only have settings for speed (RPM), so calculation of centrifugal force and control of plasma residual platelets seems important.

Aims: To evaluate the influence of residual platelets on Prothrombin Time (PT), Activated partial thromboplastin time (APTT), FV, FVIII and FIX measured in fresh and postfreeze-thaw plasma samples after 15 days at -20°C .

Methods: Blood was collected from 11 healthy donors. Different tubes were centrifuged 10 min at [RPM (g)]: 3000(1600), 1500 (400) and 1000 (200). After centrifugation, plasmas were separated in 2 aliquots: one processed within 4 h (fresh) and the other frozen at -20°C for 15 days (stored). Platelets counts were assessed in all aliquots.

Results: In fresh samples platelet counts centrifuged at 1600 g were significantly lower than at 400 and 200 g. In samples centrifuged at 400 and 200 g platelet counts decreased in post freeze-thaw samples compared to fresh ($P = 0.03$) due to the breakdown of them during freezing cycle.

Residual platelets in fresh samples after 400 or 200 vs. 1600 g centrifugation, did not affect the parameters except a slight APTT shortening ($P = 0.04$).

Coagulation results in fresh and stored plasmas centrifuged at 1600 g did not change.

In postfreeze-thaw plasma (400 vs. 1600 g), we observed PT ($P = 0.008$) and APTT ($P = 0.018$) prolongations, lower levels of FVIII ($P = 0.008$) and FIX ($P = 0.004$), with a tendency of lower FV ($P = 0.089$). Comparing postfreeze-thaw plasmas obtained at 200 vs. 1600 g, we observed prolongation of PT ($P = 0.011$) and lower levels of FV ($P = 0.006$), with a tendency in FVIII.

As the speed of centrifugation decrease and more residual platelets are, clotting factors decrease causing prolongations of PT and APTT in postfreeze-thaw plasmas.

Conclusion: Centrifugation at 1600 g for 10 min is suitable for plasma processing fresh or postfreeze-thaw after for 15 days at -20°C .

Disclosure of Interest: None declared.

PO080-TUE

Correlation to FVIII:C in two thrombin generation assays: TGA CAT and innovance ETP

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Background: Several thrombin generation tests are available but few have been directly compared. A considerable inter-lab variation within the same method has also been observed.

Aims: To investigate the correlation of two thrombin generation tests (TGT), thrombin generation assay-calibrated automated thrombogram (TGA-CAT) and INNOVANCE ETP, to FVIII: C in a group of patients with hemophilia A. A secondary aim was to investigate inter-lab variation within the TGA-CAT method.

Methods: Blood samples were taken from 45 patients with mild, moderate and severe hemophilia A. The laboratory analyses were performed at Center for Coagulation, Skåne University hospital, Malmö, Sweden and Department of Clinical Chemistry, Karolinska University hospital, Stockholm, Sweden. The TGA-CAT method was performed at both centers while the INNOVANCE ETP was only performed at the Stockholm center. Correlation between parameters was evaluated using Spearman's rank correlation test. For determination of the TGA-CAT inter-lab variability Bland-Altman, 45 degree scatter plot and frequency plot were used.

Results: Correlation between the TGA-CAT parameter ETP and the INNOVANCE ETP was poor ($R = 0.546$). When FVIII concentrations were grouped as severe, moderate and mild hemophilia and plotted against ETP values a considerable overlap between the groups was seen with both assays. Correlation of ETP to FVIII: C was similar between the two methods (0.734 and 0.701 respectively). The variability of the TGA-CAT results performed at the two centers was reduced after normalization (before normalization only 29% of values showed less than 10% difference, while after normalization the number increased to 41%).

Conclusion: The parameter ETP of the two TGT: s correlated poorly to each other but showed a comparable correlation to FVIII:C. Normalization with normal plasma reduced the inter-lab variation in the TGA-CAT method, but remained at high levels. Further improvement of standardization of these methods is warranted.

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PO081-TUE

Influence of various concentrations of anticoagulants on the thrombodynamics assay: an *in vitro* study

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Background: The impact of new anticoagulants on classical coagulation tests is poorly related to their dosage. Global hemostasis assays as the new Thrombodynamics assay allows real-time visualization of *in vitro* clot growth from the surface of tissue factor.

Aims: The aim of this study was to evaluate the impact of different anticoagulants (LMWH, dabigatran, apixaban and rivaroxaban) on Thrombodynamics assay (TD) in comparison to Thrombin Generation (TG) and routine coagulation tests.

Methods: 34 plasma samples were evaluated in parallel using TD, TG and routine coagulation tests: 10 plasmas of normal subjects and 24 plasmas spiked with various concentrations of different anticoagulants. Sensitivity of coagulation tests to anticoagulants was established using the concentration of each drug needed to double or halve the basal value for each parameter (C/2 or 2C).

Results: LMWH: Highest sensitive parameters were Velocity (Vel), Endogenous Thrombin Potential (ETP) and Peak (P) of TG 1pM and Vel of TD for heparin concentrations between 0.19 UI mL^{-1} and 0.25 UI mL^{-1} . TG curves using 5pM showed a nice dose-response relationship with increasing LMWH concentrations. Apixaban: The most sensitive parameter was the Vel in TG 5pM with a C/2 of 46 ng mL^{-1} while in TD Lag Time (Tlag) doubled with C2 of 170 ng mL^{-1} . Rivaroxaban: Most sensitive parameters were Tlag, Vel, Time to Peak (TTP) and P of TG 1pM for heparin concentrations between 31 and 50 ng mL^{-1} and Tlag of TD with C/2 of 34 ng mL^{-1} . TG curves using 5 pM showed a dose-effect relationship with increasing concentrations of rivaroxaban. Dabigatran: Tlag of TD doubled for a concentration of 42 ng mL^{-1} , and Thrombin Time (TT) in routine test for a very similar concentration of 55 ng mL^{-1} .

Conclusion: Our data showed that TG 5pM offered the best dose-effect response to increasing concentrations of anticoagulants. In this particular application, TD didn't offer any benefit compared to TG neither improved the understanding of the mechanism of action of each drug.

Disclosure of Interest: None declared.

PO082-TUE

Overall hemostatic potential assay is sensitive to different plasma freezing techniques

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Background: Plasma sample freezing is routinely performed in coagulation laboratories, but freezing techniques are not standardized. Global assays, such as overall hemostatic potential (OHP), that can reliably detect hyper- and hypocoagulable states, may be particularly sensitive to preanalytical conditions, such as freezing.

Aims: To test the influence on OHP of three commonly used freezing techniques.

Methods: Citrated plasma samples ($n = 92$) were aliquoted and frozen in three different ways: the first aliquot was put directly into freezer at -20°C , the second was put directly into freezer at -70°C , and the third was snap frozen in liquid nitrogen and then stored at -70°C . After 43 ± 2 days samples were thawed (5 min at 37°C), thoroughly mixed and analyzed. OHP with overall coagulation potential (OCP) and overall fibrinolytic potential (OFP) as supplementary parameters was determined spectrophotometrically by repeated fibrin formation and degradation registration in two parallel plasma samples. To the first sample thrombin was added (OCP) and to the second sample both thrombin and tissue-type plasminogen activator (OHP) were added. OFP was calculated as $[(\text{OCP}-\text{OHP})/\text{OCP}] \times 100$ (%). Repeated measures ANOVA was calculated with the Bonferroni's multiple comparison test.

Results: OHP (17.6 ± 9.4 Abs-sum) and OCP (27.4 ± 9.8 Abs-sum) of -20°C plasma were significantly lower compared to -70°C plasma (18.5 ± 9.5 and 29.3 ± 9.4 Abs-sum, respectively) and liquid nitrogen plasma (18.2 ± 9.5 and 28.8 ± 9.7 Abs-sum, respectively, all $P < 0.001$), while no effect of snap freezing in liquid nitrogen was noted compared to freezing directly at -70°C . Different freezing techniques had no effect on OFP.

Conclusion: Our study showed significantly lower OHP and OCP in samples stored at -20°C . On the other hand, snap freezing in liquid nitrogen was redundant when samples were stored at -70°C . Different freezing techniques should be taken into account when interpreting OHP results.

Disclosure of Interest: None declared.

PO083-TUE

Defining the reference range for blood clot gel point and fractal dimensionD'silva L¹, Alikhan R², Stanford S¹, Sabraa A¹, Lawrence M¹, Williams R³ and Evans PA¹¹College of Medicine, Swansea University, Swansea;²Haemophilia and Thrombosis Centre, University Hospital of Wales, Cardiff; ³College of Engineering, Swansea University, Swansea, UK

Background: A rheological method has been developed to determine the viscoelastic properties of the incipient blood clot, allowing measurement of biomarkers: fractal dimension (Df) and clot formation time (TGP) calculated from the gel point. Elevated levels of these biomarkers have previously been described in patients with acute venous and arterial thrombotic events and similarly, *in vitro* modelling suggests reduced biomarker levels in the presence of haemostatic failure.

Aims: The potential role for these biomarkers to predict the risk of thrombotic events or haemostatic failure is limited by the lack of a defined normal level in adults with no concurrent illness and there is a clear need to define a healthy index for these parameters.

Methods: Healthy adult volunteers identified with no personal/family history of bleeding or thrombotic disorder; no acute/chronic infective,

inflammatory or malignant condition; no renal/liver dysfunction and no anticoagulant or antiplatelet medication. Standard kinetic haemostasis markers (PT, APTT, fibrinogen) were measured. In addition whole blood was loaded into a double-gap concentric cylinder of a TA instruments AR-G2 controlled-stress rheometer, to measure TGP and Df.

Results: We tested 141 healthy volunteers, age 21–94 (67 males, mean age 54 ± 21 years, 74 females, mean age 51 ± 19 years). There was correlation between age and fibrinogen ($r = 0.44$, $P = 0.0005$), but no correlation between age and the biomarkers (TGP and Df). Similarly there was no correlation between PT, APTT, fibrinogen and the biomarkers. The fractal dimension for healthy blood was 1.73 (95% CI: 1.72–1.74).

Conclusion: A fractal dimension of 1.7 is reproducibly displayed in nature from snowflakes to bacterial reproduction. We describe for the first time a healthy index for blood fractal dimension, which can be used as a point of reference in future viscoelastic biomarker studies exploring haemostasis failure or predicting arterial/venous thrombosis risk.

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PO084-TUE

Effect of hypothermia and hemodilution on clotting activation: rotational thromboelastometry analysis in an artificial environment from healthy subjectsBoraso S¹, Gagliardi G¹, Agnoli A¹, Bettin C¹, Carraro P², Afshar H², Lembo G³, Prisco D³ and Panicia R³¹Intensive Care Unit; ²Clinical Pathology Laboratory, Sant'Antonio Hospital, Padua; ³Experimental and Clinical Medicine, University of Florence, Florence, Italy

Background: Moderate accidental hypothermia and hemodilution are common finding in bleeding patients.

Aims: This study was aimed to evaluate single and combined effect of the hemodilution and hypothermia (34°C) on clot formation *in vitro* by using the rotational thromboelastometry by ROTEM device (TEM Int, Germany).

Methods: Citrated whole blood was taken from 11 healthy volunteers. Forty-four samples were obtained that were split into 4 blocks: undiluted and diluted (50% Ringer lactate) analyzed at 37°C and undiluted and diluted (50% Ringer lactate) analyzed at 34°C . After dilution, normal values of pH and electrolytes (especially Ca^{++}) were verified by hemogasanalysis. To investigate the effect of hypothermia, samples were incubated in a water tank for 40 min at 34°C . For ROTEM analysis INTEM test (intrinsic activation), EXTEM test (extrinsic activation) and FIBTEM test (extrinsic pathway activation without the contribution of platelets) were performed. For each test the following parameters were recorded: CT (clotting time), CFT (clot formation time), αA (α -angle), MCF (maximum clot formation), ML (maximum lysis).

Results: By itself hypothermia significantly impaired CFT (80 ± 15 s, $P < 0.0001$); αA ($75 \pm 3^{\circ}\text{C}$, $P = 0.006$) in INTEM test; CFT (96 ± 34 s, $P = 0.02$), αA ($71 \pm 6^{\circ}\text{C}$, $P = 0.005$) in EXTEM test. Hemodilution affected CFT (175 ± 42 s, $P < 0.0001$), αA ($65 \pm 6^{\circ}\text{C}$, $P < 0.0001$), MCF (48 ± 5 mm, $P < 0.0001$) in INTEM test; CFT (133 ± 30 s, $P = 0.0002$), αA ($68 \pm 7^{\circ}\text{C}$, $P = 0.002$), MCF (47 ± 7 mm $P < 0.0001$) in EXTEM test; CT (72 ± 26 s, $P = 0.02$), MCF (7 ± 2 mm, $P = 0.0002$) in FIBTEM test. Considering the hemodilution/hypothermia interactions, CFT was found prolonged in INTEM ($P = 0.02$).

Conclusion: The variables considered significantly influence clot formation but by different ways. The hypothermia would act slowing the

kinetics of clot formation, hemodilution affects stability firmness and strength of clot. Hemodilution/hypothermia interactions produce a slowdown in the kinetics of the formation of a stable clot.

Disclosure of Interest: None declared.

PO085-TUE

Test specific assessment of effects of haemolysis on citrated blood samples using Sysmex CS series analysers

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Background: Haemolysis during blood sample collection and processing is a frequent occurrence in citrated blood samples which can significantly alter results of some clotting tests making detection and rejection an important requirement to ensure patient safety. Haemolysis in samples is automatically detected by some Sysmex CS series analysers with user definable alert flags using a scale of 1 to 5 indicating increasing levels of haemoglobin (Hb) in plasma

Aims: To assess test specific thresholds for effects of haemolysis on clotting tests.

Methods: Freeze thaw lysed red cells were added to plasma to establish the level of free Hb required to generate flags of 1 to 5. Rejected haemolysed patient samples ($n = 20$) were analysed alongside matched samples from the same subjects collected within 4 h of the reject sample and which lacked detectable haemolysis. In addition a series of plasma pools were spiked with haemoglobin. The flag level required to alert when a test result was altered by $> 10\%$ (Antithrombin, Protein C) or $> 15\%$ (other tests) as a consequence of haemolysis was assessed for a series of tests. All reagents were from Siemens Healthcare Diagnostics.

Results: The level of haemoglobin causing $> 15\%$ changes was different in spiked samples compared to genuine patient samples for some tests. DDimer FXIII and APTT were more tolerant of haemolysis in spiked samples. Taking all data into account the following flag levels were required to ensure $< 15\%$ change in results ($< 10\%$ for AT,PC) with equivalent plasma Hb in brackets: level 5 ($> 300 \text{ mg dL}^{-1}$) – PT (Thromborel S, Clauss Fib, TCT); level 4 ($240\text{--}300 \text{ mg dL}^{-1}$) PT (Innovin), PC; level 3 ($180\text{--}240 \text{ mg dL}^{-1}$) – AT; Level 1 ($40\text{--}120 \text{ mg dL}^{-1}$) – APTT (Actin FS, Actin FSL), FXIII, DDimer

Conclusion: The level of haemolysis causing clinically important changes is test specific so test specific thresholds should be used to assess patient samples which have been haemolysed during collection/processing. Assessment of haemolysis interference should include patient samples.

Disclosure of interest: A. Woolley: None Declared, Y. Tabuchi Employee of: Sysmex, S. Kitchen Speaker Bureau of: Sysmex

PO086-TUE

Province wide implementation of the thrombin time for qualitative assessment of dabigatran clearance

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Background: The PT and PTT are too insensitive to dabigatran to be useful in assessing for drug clearance. Conversely, specialized testing is often impractical or has too long of a turn-around time (TAT). Stroke patients on dabigatran are paradigmatic of the problem as testing must

be both sensitive to the drug and have a rapid TAT in order to guide therapy.

Aims: To evaluate whether Thrombin Time (TT) could be implemented at all stroke management sites in Alberta (Canada) with a 1 h TAT that would: (i) reliably differentiate dabigatran levels $> 30 \text{ ng mL}^{-1}$; (ii) demonstrate sufficient inter-lab and lot consistency to allow standardization.

Methods: Initially, each lab validated its own TT assay for general use. This was followed by a single point dabigatran calibration. Given that the TT can be shortened by high fibrinogen levels, we used 10 normal patient plasma samples spiked *in vitro* with 20 ng mL^{-1} of drug per analyzer tested. A TT cut-off was calculated for each reagent-analyzer peer group as the pooled mean-2*SD rounded to the nearest 5 s. Each lab was then sent two External Quality Assurance (EQA) samples prepared from a variety of patient plasmas with normal INR, PTT and TT but high fibrinogen ($5.0\text{--}8.9 \text{ g L}^{-1}$), spiked with 35 ng mL^{-1} of drug.

Results: There were 23 labs with 33 analyzers, all of which implemented the TT. Cut-off values were established as follows (a=analyzers, L=reagent lot #'s tested): Diagnostica Stago reagent on STA-R (a = 2, L = 2) or Compact (a = 3, L = 1) = 40 s; Siemens Test Thrombin on Sysmex CA-Series (a = 10, L = 6) = 35 s; IL Hemosil (5 mL) reagent on ACL TOP series (a = 8, L = 2) = 35 s; IL Hemosil reagent on ACL Elite Pro (a = 7, L = 2) or ACL 1000 (a = 3, L = 2) = 30 s. All EQA samples ($n = 60$) gave a TT result above the cut-off. In contrast, only 54% ($n = 52$) gave an abnormal PTT. Inter-lab/lot variation was low.

Conclusion: A single point calibrated TT can be used to qualitatively assess for dabigatran clearance using a variety of reagents/analyzers.

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PO087-TUE

Effects of rivaroxaban on PT and APTT as determined with a full range of reagents: results of a UK NEQAS blood coagulation exercise

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Background: PT and APTT reagents vary in their sensitivity to rivaroxaban. Guidelines recommend that laboratories should be aware of the sensitivity of their coagulation screening tests to the presence of rivaroxaban. Data are needed on the full range of PT/APTT reagents in routine use.

Aims: To assess the effects of a range of rivaroxaban concentrations encompassing peak/trough/accumulation levels on prothrombin time (PT) and activated partial thromboplastin time (APTT) as determined by all reagents in use by participants in UK NEQAS Blood coagulation

Methods: The same pooled normal plasma was spiked with 0, 29, 121, 346 and 734 ng mL^{-1} rivaroxaban (provided by Bayer), lyophilised in 1 mL aliquots and despatched to PT/APTT participants in the UK National external Quality Assessment Scheme (NEQAS) for Blood Coagulation. Rivaroxaban concentrations were determined by anti Xa/mass spectrometry. The median PT or APTT ratio for each reagent group was calculated (test/mid point normal range). Results were received from > 600 centres ($\sim 60\%$ UK and 40% non UK)

Results: 20 different PT and 18 different APTT reagents were used. The sample containing no rivaroxaban had normal PT and APTT as expected. PT Results

29 ng mL^{-1} – all reagent medians normal or near normal; 121 ng mL^{-1} all medians prolonged (range 1.25–1.81); 346 ng mL^{-1} –

range 1.50–2.97; 734 ng mL⁻¹ – range 2.07–5.45. Innovin and Thromborel S were the least sensitive reagents. Neoplastin R was the most sensitive. APTT Results

29 ng mL⁻¹ – some medians were normal and others prolonged – range 1.08 – 1.33; 121 ng mL⁻¹ – all medians prolonged, range 1.33–1.67; 346 ng mL⁻¹ – range 1.63–2.10; 734 ng mL⁻¹ – range 2.16–2.94.

Conclusion: A concentration of 121 ng mL⁻¹ (below average expected peak levels) prolonged PT and APTT with all reagents. Prolongation of APTT and especially PT by higher rivaroxaban concentrations varied markedly between reagents so interpretation of PT and APTT results in the presence of rivaroxaban must take account of the reagents used.

Disclosure of Interest: S. Kitchen Speaker Bureau of: Bayer, I. Jennings: None Declared, R. Jones: None Declared, D. Kitchen: None Declared, T. Woods: None Declared, I. Walker: None Declared

PO088-TUE

A novel plasma-based Factor Xa generation assay defines interaction between FVIIIa and each component of XASE complex in mild hemophilia A.

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Background: In mild/moderate hemophilia A (MHA), FVIII activity (FVIII:C) by one-stage assay may not evaluate the actual hemostatic potential of innate FVIII(a). We focused on FXa generation (XaG) following to the formation of FVIIIa/FIXa/FX on the phospholipid (PL) as representative of hemostatic function of FVIIIa. We reported MHA cases carrying such F8 mutation(s) as p.T693I impairing the FIXa association (ISTH2013), p.R1800H enhancing the FX association (TH2013) and p.R612C with attenuated PL binding (ASH2014).

Aims: We attempted to establish a plasma-based XaG assay to determine the clinical severity and its mechanism caused MHA.

Methods: XaG assay was performed using plasmas initiated by thrombin (0.01 nM) with FIXa (0–5 nM), FX (0–200 nM), PL (0–114 µM) and S-2765. The standard plasmas consisting of FVIII-deficient plasma and serially diluted rFVIII (0.2–100 IU dL⁻¹) were prepared. Each XaG value was evaluated by Michaelis-Menten kinetics equation.

Results: XaG in PNP was equivalent to that of FVIII 100 IU dL⁻¹, whilst XaG in FVIII deficient plasma from severe HA was not detected (sensitivity 0.2–100 IU dL⁻¹). XaG was examined in 5 cases with MHA carrying p.T693I (Case 1 [FVIII:C 30 IU dL⁻¹] severe phenotype), p.R1800H (Case 2 [4.8 IU dL⁻¹] mild, Case 3 [3.5 IU dL⁻¹] mild), and p.R612C (Case 4 [9.7 IU dL⁻¹] mild, Case 5 [5.8 IU dL⁻¹] mild). XaG in Case 2–5 were equal to control plasma with same levels of FVIII:C, whilst Case 1 showed ~50% reduction of XaG compared to control, equivalent to that of FVIII:C 10 IU dL⁻¹ in standard samples. Case 1 showed reduced affinity for FIXa (K_m^{app} 5 ± 1.5 nM) compared to control (1.2 ± 0.3 nM). Case 2, 3 facilitated higher affinity for FX (7.2 ± 1.3 nM) than control (24 ± 7.3 nM), and Case 4, 5 showed ~50% reduced affinity for PL (3.4 ± 0.8 µM) compared to control (1.5 ± 0.3 µM).

Conclusion: Our plasma-based XaG assay defined and quantified the potential of interaction between FVIIIa in MHA and FIXa, FX and/or PL. This assay will make it possible to characterize the hemostatic function of FVIIIa in MHA.

Disclosure of Interest: None declared.

PO089-TUE

The FVIII plasma activity of rVIII-SingleChain can be measured in both the one-stage and chromogenic substrate assays

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Background: rVIII-SingleChain is a B-domain truncated, single-chain recombinant factor VIII molecule. Activity measurements exhibit a discrepancy between the chromogenic substrate (CS) and one-stage (OS) assay formats. PK evaluation of rVIII-SingleChain in 27 male subjects, revealed a strong linear relationship between the CS and OS assay results, allowing for reliable interpretation of either method.

Aims: An international, multicenter, randomized and blinded field study, designed to determine the inter-laboratory variability of measurements of both rVIII-SingleChain and Advate[®] across assay methods was initiated.

Methods: Plasma samples were spiked at 0.04, 0.3, 0.6 and 1.0 IU mL⁻¹ for rVIII-SingleChain and Advate[®], blinded, and distributed to local laboratories to be assayed by the OS and CS assays. Laboratories were instructed to follow their routine laboratory practices and use their own routine in-house FVIII standard, FVIII-deficient plasma and assay reagents. A Standard Human Plasma standard was included in the sample kit, to allow for recalculation of results against a common standard as well as a product specific standard.

Results: Preliminary results suggest intra and inter laboratory variability in OS assays were similar for rVIII-SingleChain and Advate[®]. For rVIII-SingleChain, the OS assay values underestimated target values at all spiked concentrations. In contrast, the CS assays were closer to the target value for rVIII-SingleChain.

Conclusion: Preliminary data shows a consistent underestimation of rVIII-SingleChain FVIII:C when using the OS assay. Due to the consistency and reproducibility, this underestimation is predictable, suggesting that the activity of rVIII-SingleChain from patient samples can be reliably measured with both the CS and OS assay methods. These results support an approach to provide clinical guidance on interpretation of FVIII:C measurements when the OS assay is used for monitoring.

Disclosure of Interest: K. St.Ledger Employee of: CSL Behring, A. Feussner Employee of: CSL Behring, U. Kalina Employee of: CSL Behring, C. Horn Employee of: CSL Behring, H. Metzner Employee of: CSL Behring, A. Stowers Employee of: CSL Behring, D. Bensen Kennedy Employee of: CSL Behring.

PO090-TUE

Usefulness of coagulation screening tests for diagnosis of coagulopathy

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Background: Coagulation screening tests represented by prothrombin time (PT) and activated partial thromboplastin time (aPTT) provide rapid but non-specific information on potential haemostatic defects and are frequently used to diagnose a bleeding disorder.

Aims: To evaluate the utility and reliability of PT and aPTT to diagnose coagulation defects.

Methods: All patients evaluated between October 2011 and July 2014 with at least an abnormal result of PT or aPTT were enrolled. They were investigated with the MCMDM-1 VWD bleeding questionnaire

and by performing 1st-level screening laboratory tests (platelets count, PT, aPTT, fibrinogen and coagulation factors functional dosages). In patients with bleeding diathesis 2nd- and 3rd-level laboratory tests were also performed (von Willebrand factor activity, platelet function studies, fibrinolysis assessment and factor XIII activity).

Results: 240 patients (134 males) aged 5 months-81 years (median 18 years) were included. Sixty-five patients (27%) were referred for evaluation of a prolonged PT, 140 (58%) for a prolonged aPTT and 35 (15%) for a prolonged PT and aPTT. A family history of bleeding tendency was ascertained in 20 patients (8%) and a liver disease in 5 (2%). The median BS was 1 (range -3 to 21) with a significant value (≥ 5) detected in 26 patients (11%). An abnormal PT and/or aPTT was confirmed in 175/240 patients (73%), with 51/175 (29%) prolonged PT, 97/175 (56%) prolonged aPTT and 27/175 (15%) prolonged PT and aPTT. A coagulation defect was diagnosed in 177/240 patients (74%) including 6 severe, 8 moderate and 150 mild deficiencies. First-level laboratory tests were able to diagnose 140/177 defects (79%), while 2nd- and 3rd-level laboratory tests were necessary for detecting 5/177 (3%) and 32/177 (18%) defects, respectively.

Conclusion: PT and aPTT provide general information on the presence of an underlying coagulation defect, but our data confirm they could miss mild abnormalities, diagnosis of von Willebrand disease and platelet function disorder.

Disclosure of Interest: None declared.

PO091-TUE

Bleeding symptoms or bleeding disorder? That is the question

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Background: The diagnosis of underlying bleeding disorders in patients with bleeding symptoms still represents a significant challenge.

Aims: To assess the prevalence of bleeding disorders in patients with bleeding symptoms.

Methods: All patients referred to our Center between October 2011 and July 2014 for bleeding diathesis were enrolled. They were investigated with the MCMDM-1 VWD bleeding questionnaire and by performing 1st-level screening laboratory tests (PLTs count, PT, aPTT, FBG and coagulation factors functional dosages). In patients with bleeding diathesis 2nd- and 3rd-level laboratory tests were also performed (VWF activity, PLT function studies, fibrinolysis assessment and FXIII activity). Any bleeding event that required the use of either anti-haemorrhagic drugs or surgical haemostasis was considered as severe.

Results: 258 patients (79 males) aged 6 months-92 years (median 39 years) were included. Family history of bleeding tendency was ascertained in 29 patients (11%). The median BS was 3 (range -2 to 23) and the most frequent bleeding symptoms were bruising (44%), menorrhagia (38%) and epistaxis (21%). Sixty-four patients (25%) had a BS ≥ 5 and 105 (41%) experienced at least one severe bleeding symptom. Overall, 5 severe and 41 mild haemostatic defects were diagnosed in 46 patients (18%) with a median BS of 4 (range 0 to 10). Among these, 18 (39%) had a BS ≥ 5 and 27 (59%) have had at least one severe bleeding symptom. One hundred eighty patients (70%) who underwent all the aforementioned tests had no diagnosis of bleeding disorders. The median BS in this group was 3 (range -2 to 18) with a BS ≥ 5 in 34 (19%) and the presence of at least one severe bleeding symptom in 67 (37%).

Conclusion: Our results show that the majority of patients with history of bleeding symptoms has not necessary a hemostatic defect. In patients with a severe bleeding diathesis further evaluation using next

generation sequencing might help to understand the role of novel genes encoding for unknown proteins involved in haemostasis.

Disclosure of Interest: None declared.

PO092-TUE

Evaluation of an automated coagulometer in a new haemophilia care center

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Background: Implementation of a new coagulometer requires a proper validation, particularly in high complex laboratories.

Aims: To evaluate ACL TOP 300 coagulometer performance in terms of precision and accuracy in a NHCC in Argentina.

Methods: CLSI protocols (EP 15-A2 and H57A) were applied. Within-run precision (WR), and within-laboratory precision (WL) on Prothrombin Time (PT) and activated partial thromboplastin time (APTT) were evaluated by using three levels of controls (Instrumentation Laboratory, IL): Low (L), Normal (N) and High (H). Each control was processed ten times to determine WR, and in triplicate for five days to determine WL. 80 samples of healthy donors, patients under oral anti-coagulants Anti vitamine K, factor deficiencies and lupus anticoagulant were used for instruments comparison. At least 20 samples were analyzed to compare Factor and Fibrinogen dosages. APTT R of 40 samples from patients with hemophilia was compared separately. EP evaluator was used for statistical analysis. Instruments: ACL-TOP 300 (IL) and STA COMPACT (STAGO) as reference. Reagents from IL or STAGO were used, respectively.

Results: Observed value/manufacturer requirement were: %CV WR: L: PT 1.3/2.2, APTT 2.8/2.4; N: PT 0.7/1.1, APTT 1.2/1.1; H: TP 2.4/2.5, APTT 3.0/2.9. %CV WL: L: PT 1.4/3.3, APTT 1.8/3.8; N: PT 1.7/2.5, APTT 1.9/2.5; H: PT 2.7/3.0, APTT 2.9/4.9. Correlation between equipments (Pearson Correlation Coefficient, r): PT 0.99, INR 0.99, APTT R 0.99, hemophilia A APTT R 0.92; concordance 0.84; hemophilia A APTT R with APTT R < 2.9 (n: 34): 0.95; concordance 0.95, Fibrinogen 0.99, FII 0.99, FV 0.95, FVII 0.98, FVIII 0.98, FIX 0.98, FX 0.99.

Conclusion: Manufacturer requirements were achieved for PT and APTT WL, but WR requirements were not achieved for APTT. Correlation between ACL-TOP 300 and STA COMPACT was successful for all parameters, except samples from hemophiliacs with APTT R > 2.9, which show lower concordance.

Disclosure of Interest: None declared.

PO093-TUE

Factor VIII inhibitors quantified using clot waveform analysis

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Background: Clot waveform analysis (CWFA) extends the interpretation of aPTT measurement curves. We postulate that the first, second and third derivative of the fibrin generation curve represents thrombin, prothrombinase and tenase complex activities. Inhibitor patients show diminished clot formation that should be reflected in CWFA.

Aims: We tested the feasibility of CWFA in monitoring inhibitors in patients with haemophilia A (HA) and B (HB). The CWFA parameters were expressed in nm thrombin or respective derivatives. Inhibitors

based on this method were compared with established methods like Bethesda (BE) or Nijmegen.

Methods: CWFA was performed using Synthasil as activator. The derivatives were calculated using the Savitzky-Golay algorithm and calculated as thrombin concentration (nM) or thrombin formation (nM s^{-1}). All inhibitor patients were diagnosed in our centre.

Results: All inhibitors that were diagnosed with the established methods were also recognized in the CWFA-based assay. The absolute values are lower because CWFA reflects more the overall coagulation capacity and not only one factor. Patients monitored over a long time show comparable kinetics in CWFA and BE. This is true for FVIII and FIX inhibitors and low and high responders. Patients without any inhibitors are negative in BE and CWFA based calculations. The variation coefficients (CV) are comparable.

Conclusion: CWFA is a cheap and easy method to monitor patients with inhibitors and can be done without any additional measurements beside an aPTT. The correlation between CWFA and Bethesda/Nijmegen based inhibitors is excellent. The method is cheap and simple, but needs the transformation from optical densities to thrombin concentrations. Based on CWFA analysis inhibitors can be recognized not only in specialized labs, inhibitors can be diagnosed in all labs that can measure an aPTT.

Disclosure of Interest: None declared.

Cancer and thrombosis / hemostasis II

PO094-TUE

Tissue factor downregulation by the putative oncogenic microRNA, MIR-520G, in embryonal brain tumour cells

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Background: Tissue factor (TF) acts as a receptor for coagulation factor VIIa and mediates both clotting and proangiogenic intracellular signals. Oncogenes deregulate tumour cell interactions with the vascular system including the expression, activity, and signaling properties of TF. Embryonal tumours with multilayered rosettes (ETMR) are pediatric brain lesions that demonstrate a unique genomic amplification of the chromosome 19 microRNA (miR) cluster (C19MC) leading to the overexpression of related miRs including miR-520 g.

Aims: To determine whether miR-520 g is part of the oncogenic circuitry that controls TF resulting in perturbations of vascular homeostasis and increased disease aggressiveness.

Methods: We have used immunodetection of TF and fibrin in C19-amplified and non-amplified tumour tissues. In addition, PCR, western blotting, and activity assays were used to detect the linkage between miR-520 g and TF in cultured cells.

Results: Decreased fibrin content paralleled reduced TF protein in ETMR. This effect was recapitulated in cells overexpressing miR-520 g, which exhibit lower TF protein and emission as EVs. MiR-520 g specifically and directly targets the TF 3'UTR. Reduced TF protein and elevated miR-520 g are associated with decreased procoagulant activity and signaling. Upregulation of endogenous miR-520 g in stem-like growth conditions is associated with decreased TF protein.

Conclusion: In spite of the absence of clinically manifest thrombosis, pediatric brain tumours interact with the coagulation pathway. Ours is the first study that examines this as a function of TF downregulation

by miR-520 g, a putative oncomir in ETMR. Given that classical oncogenes often upregulate TF, the significance of this observation is puzzling and may be linked to the unique stem cell-rich nature of ETMR. We propose that oncomirs may control the brain tumour coagulome.

Disclosure of Interest: None declared.

PO095-TUE

VTE recurrence and bleeding in patients with cancer.

Results of the Optimev study

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Background: VTE treatment in patients with cancer is still challenging in clinical practice despite therapeutic advances and international guidelines.

Aims: To evaluate VTE recurrence and bleeding risk, we have analysed the data of the Optimev study.

Methods: Study type was a exposed/non exposed one nested in a cohort study. All patients with VTE and known cancer at inclusion (exposed ones) and patients with VTE without known cancer at inclusion and during follow-up (non exposed ones) were included with a 1/2 ratio. Patients were paired depending on age, sex and VTE type.

Results: 336 patients VTE+ and cancer were compared to 672 patients VTE+ and without cancer. Criteria for analysis were VTE recurrence (DVT and/or PE), major bleeding, and death during 3 years of follow-up. Both groups had equivalent repartition of VTE type (SVT 10%, distal DVT 27%, proximal DVT 38%, PE with or without DVT 25%). Mean age was 67 (53% of men in both groups) risk of VTE recurrence was 10.6% at one year and 16.4% at 3 years in the cancer group vs. 3.4% and 9% in the non exposed group ($P < 0.01$, HR = 2.1[1.4–3.2]). Distal DVT had the higher risk of recurrence (20% at 3 years with an anticoagulant treatment duration of 3 months). Major Bleeding risk was 5.9% at one year and 8.9% at 3 years in the exposed group vs. 2.3 and 3.6% in the non exposed group ($P < 0.01$, HR=2.5[1.35–4.5]). Mortality at 3 years was 60% in the cancer group vs. 10% in the non exposed group. In comparable mortality rates, duration of anticoagulant treatment were not longer in patients with cancer. Use of long term treatment with LMWH were achieved in 30% of patients. (cohort initiated before 2010). Our result confirm the difficulty to treat patients with VTE and cancer enhancing the balance between recurrence and bleeding risk.

Conclusion: VTE treatment in patients with cancer is an important clinical problem: a high risk of recurrence but also a significant risk of bleeding were noted in our study

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PO096-TUE

Collaborative efforts of the national heart, lung, and blood institute and the national cancer institute in cancer and thrombosis

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Background: Thrombosis is a leading cause of death in cancer patients, causing delay/discontinuation of therapy, higher consumption of health care resources, and decreased quality of life. In spite of the clinical significance, research to target these issues is limited across the National Institutes of Health. Exemplified by fiscal year 2014, only 15 unique studies addressing the cancer and thrombosis interface were supported by the National Heart, Lung, and Blood Institute (NHLBI) or the National Cancer Institute (NCI), representing just 0.15% of those institutes' total grants.

Aims: Motivated by these concerns, the NHLBI and NCI are working collaboratively to identify barriers in the field and prioritize research directions.

Methods: In August 2014, the NHLBI/NCI hosted a working group on 'Cancer and Thrombosis,' bringing together scientists and clinicians in cancer research, oncology, and thrombosis and hemostasis, as well as representatives from the Food and Drug Administration and the Department of Veterans Affairs. The meeting goals were to: (i) discuss current and emerging data, (ii) identify key research questions, and (iii) generate recommendations for the most promising directions of research in cancer and thrombosis.

Results: Meeting participants emphasized the urgency and need for collaborative efforts to address cancer-related thrombosis. Recommendations included additional mechanistic studies of thrombosis in cancer; identification and validation of improved, actionable thrombotic biomarkers in cancer patients; and prospective cohort studies and intervention trials. Building on the working group discussions, a Request for Information (RFI) was issued regarding the effect of pre-analytical variables on biomarkers of thrombosis in cancer patients; the results are forthcoming.

Conclusion: Recommendations from the research community are being used to guide future directions of the NHLBI/NCI to advance cancer-associated thrombosis research for the overall benefit of cancer patients.

Disclosure of Interest: None declared.

PO097-TUE

Patients experiences of living with cancer associated thrombosis: the pelican study

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Background: Thrombosis remains the commonest preventable cause of mortality in cancer patients receiving chemotherapy. Whilst the prophylaxis and treatment of this condition is well understood, the patient experience and subsequent behavioral factors are not.

Aims: To explore the experiences of patients diagnosed and treated for cancer associated thrombosis.

Methods: Patients receiving treatment for cancer-associated thrombosis (CAT) were interviewed about their experiences of CAT within the context of their cancer journey. Twenty interviews were transcribed and analyzed using framework analysis.

Results: The CAT journey was considered a distressing one with limited support or information in complete juxtaposition with the treat-

ment they received for their cancer. Patients felt there was little ownership for the management of CAT, which further added to distress. Chemotherapy patients were well informed about the risks of febrile neutropenia, how to recognize it and when to seek medical attention. However they had limited knowledge about CAT and received no information about the condition. Red flag symptoms suggestive of CAT were attributed to chemotherapy or the underlying cancer, resulting in delayed presentation and diagnosis to hospital.

Conclusion: CAT is a common occurrence and patients view their experiences of it within the context of their overall cancer journey. However, patients receive little information to help recognize CAT and access timely treatment on the development of symptoms. Whilst other cancer complications have clear treatment pathways, thrombosis does not appear to have been afforded the same priority. A proactive approach to increase patient awareness, coupled with established CAT pathways is likely to reduce mortality, morbidity and long-term psychological distress.

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PO098-TUE

Cancer associated thrombosis: developing consensus guidance to improve patient care and outcomes

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Background: Cancer is responsible for 18% of all cases of incident VTE. Across all patients with cancer the risk for VTE is elevated between 7 and 28-fold. There are special considerations in cancer patients who develop VTE relating to clinician awareness, management strategies and improving patient care and quality of life.

Aims: To understand the perceptions and attitudes of key stakeholders in delivering care, informing practitioners of the attitudes of their peers in order to improve patient outcomes.

Methods: A small, multidisciplinary group involved in the treatment of patients with CAT met in to define themes for future development in the management of CAT across the UK. 42 consensus statements were developed and submitted to respondents by questionnaire using a 4-point Likert scale. A modified Delphi methodology was used to review responses.

Results: An initial sample of 20 respondents returned questionnaires indicating varying levels of agreement with each statement. Statements were then revised and questionnaires were returned by a further 47 respondents working with CAT across the UK. Of the 42 statements, 33 achieved > 90% agreement (78.6%), and 41 Achieved > 66% agreement (97.6%) with one statement failing to achieve 66% agreement (2.3%).

Conclusion: Respondents clearly agree that active communication between the multidisciplinary team and the patients GP are critical in managing CAT. Support is also strong for the development of service models and integrated care pathways suggesting that the need for such measures is well understood across the UK. Such measures will ensure that appropriate guidance is applied in the treatment of each patient. Responses indicate that clarification is needed regarding the implementation of current guidance on CAT. The weakest agreement is with the assertion that the best care may be provided in the secondary care

environment, suggesting that there is a view amongst clinicians that community-based care may be useful for patients with CAT.

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PO099-TUE

Reducing clot risk in post-operative cancer patients: what is the common ground among international VTE prevention guidelines?

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Background: Venous thromboembolism (VTE) is the most common cause of death after surgery in the oncology population. Currently there are five different sets of major international guidelines on prevention of VTE in the post-operative cancer population.

Aims: This study is a comprehensive review of each of the five international guidelines, for the purpose of identifying what recommendations they have in common – and where controversy remains.

Methods: Comprehensive content analysis was performed on the five current VTE prevention guidelines published by: the National Comprehensive Cancer Network (NCCN); the American Society for Clinical Oncology (ASCO); the American College of Chest Surgeons (ACCP); the European Society for Medical Oncology (ESMO); and the International Society on Thrombosis and Haemostasis (ISTH). In addition the prognostic VTE risk factors identified in the @RISTOS Project (Agnelli et al, 2006) were applied prospectively to 100 surgical cases in the author's own facility.

Results: There is a great deal of common ground among the five sets of guidelines, especially with regard to: hospitalized cancer patients; patients with certain malignancies; cancer patients who have extensive abdominal or pelvic surgery; and patients on specific chemotherapy regimens. There is some variability in terms of absolute and relative contraindications for VTE prophylaxis. Choice of pharmacologic anticoagulant therapy is fairly uniform. Based on the author's own prospective data, most cancer patients who have major surgery do indeed meet the @RISTOS criteria for significant VTE risk.

Conclusion: Clinical guidelines tend to proliferate as clinical data are produced. Often there is common ground to guide best clinical practices. Where there is controversy, there is room for critical thinking and clinical judgement. In the meantime, basic prognostic VTE risk factors can be applied in real time to help inform the management of the post-operative cancer patient.

Disclosure of Interest: None declared.

PO100-TUE

Age-adjusted D-Dimer cut-off level increases the number of cancer patients in who pulmonary embolism can be safely excluded without CTPA imaging: the adjust-PE Cancer substudy

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Background: Patients with cancer frequently present with suspected pulmonary embolism (PE). The D-Dimer test is less useful to rule out PE in cancer patients due to a lower specificity, whereas the safety of the combination of a clinical decision rule (CDR) and D-dimer test to rule out PE in these patients is unclear.

Aims: We prospectively analysed the safety and efficacy of the age-adjusted D-dimer (defined as age₁₀ in patients > 50 years) combined with CDR for the exclusion of PE in patients with cancer.

Methods: We conducted a multicentre multinational prospective management outcome study in 19 centers in Belgium, France, The Netherlands and Switzerland, the ADJUST-PE study, to validate an age-adjusted D-dimer cut-off in patients with suspected PE. The performance of the age-adjusted D-dimer cut-off and CDR was compared between patients with and without cancer. The primary outcome was the rate of adjudicated thromboembolic events during three-month follow-up.

Results: Of the 3324 patients with suspected PE, 429 (12.9%) patients had cancer. Cancer patients were older and more often had surgery or immobilisation. The prevalence of PE was 108/429 (25.2%) in cancer patients and 522/2894 (18%) in patients without cancer, $P < 0.001$. Among cancer patients with an unlikely CDR, 27/274 (9.9%) had a D-Dimer $< 500 \mu\text{g L}^{-1}$ as compared with 19.7% using the age-adjusted D-dimer cut-off; in patients without cancer, these rates were 30.1% and 41.9%, respectively. The percentage of cancer patients in whom PE could be excluded based on CDR and age-adjusted D-dimer doubled from 6.3% to 12.6%. None of these cancer patients had a venous thromboembolic event during three-month follow-up.

Conclusion: Compared with the usual cut-off, the age-adjusted D-dimer cut-off doubles the proportion of patients with cancer in whom PE can be safely excluded by CDR and D-dimer without need for CTPA imaging.

Disclosure of Interest: D. Wilts: None Declared, G. Le Gal: None Declared, P. den Exter: None Declared, J. van Es: None Declared, M. Carrier: None Declared, B. Planquette: None Declared, H. Büller: None Declared, M. Righini: None Declared, M. Huisman: None Declared, P. Kamphuisen Grant/Research Support from: Dutch Thrombosis Foundation

PO101-TUE

Rates of venous thromboembolism despite thromboprophylaxis in multiple myeloma patients receiving lenalidomide or pomalidomide: a systematic review and meta-analysis

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Background: The use of immunomodulatory drugs (IMiDs), especially thalidomide, in combination with dexamethasone (Dex) in multiple myeloma (MM) patients has been shown to significantly increase the incidence of venous thromboembolism (VTE). Clinical practice guidelines recommend the use of thromboprophylaxis in patients receiving IMiDs for MM. However, the best type of thromboprophylaxis, especially in patients receiving more recent IMiDs (e.g. lenalidomide (LEN), pomalidomide (POM)), remains unknown.

Aims: The aim of this review was to determine the rates of VTE in MM patients receiving LEN or POM and thromboprophylaxis. The pooled rates of VTE was further characterized based on the disease status (newly diagnosed (ND) or relapsed/refractory (RR)), as well as the type of IMiD.

Methods: A systematic literature search was done using MEDLINE, EMBASE and Cochrane Library. Two reviewers independently assessed all articles identified from the initial literature search to determine their eligibility. Both reviewers independently extracted the required information from each selected study. Any discrepancies were resolved through consensus.

Results: A total of 917 patients were included in the analyses (ND MM + LEN ($n = 327$); RR MM + LEN ($n = 324$); RR MM + POM ($n = 266$)). In patients with RR MM being treated with POM and DEX, the rate of VTE while on aspirin (ASA) was 3.5 per 100 patient-years (95% CI: 1.5–6.2). In patients with ND MM treated with LEN and DEX or Prednisone (PRED), the rate of VTE while on ASA was 8.0 per 100 patient-years (95% CI: 1.3–19.6). This rate fell to 3.9 per 100 patient-years (95% CI: 2.4–5.8) when patients with RR MM were treated with this same regimen.

Conclusion: The rates of VTE in RR MM patients receiving LEN or POM are low, and thromboprophylaxis with ASA seems adequate. Rates of VTE are higher in patient with ND MM receiving LEN and DEX or PRED. Further trials assessing risks and benefits of other thromboprophylactic regimens in this patient population are warranted.

Disclosure of Interest: M. Kimpton: None Declared, M. Carrier: None Declared, J. Tay Consultant for: Celgene and Janssen, A. Davis: None Declared, G. Le Gal: None Declared

PO102-TUE

Prospective evaluation of risk assessment models and biological markers of hypercoagulability for the identification of high VTE risk patients with lung adenocarcinoma. The roadmap study

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Background: In patients with lung adenocarcinoma (LA), metastasis (MTS), advanced stage and chemotherapy (CTx) are risk factors for

thromboembolism (VTE). Routine thromboprophylaxis is not recommended but individualized risk assessment is encouraged.

Aims: The selection of the most relevant hypercoagulability biomarkers (HB) for incorporation into the risk assessment models (RAM) for VTE

Methods: Patients with documented LA eligible for CTx at distance of at least 3 months from surgery or hospitalization were included. They were either CTx naive (NG) or had received CTx (OTG). Control group (CG) consisted of 30 healthy age & sex-matched individuals. We assessed them for thrombin generation (TG), P-Selectin, heparanase (HPA), procoagulant phospholipids (PPL), factor VIIa, D-Dimers (DDi) and Tissue Factor activity (TFa).

Results: Patients showed significantly shortened PPL and higher levels of TFa, DDi and HPA as compared to the CG. FVIIa levels were lower in patients compared to CG. The NG showed significantly shorter lag-time and lower ETP as compared to the OTG. It also showed significantly higher levels of HPA as compared to the OTG. The increase of TG and of HPA, P-Selectin, FVIIa was associated with the stage. Patients with MTS had higher levels of P-Selectin, TFa, DDi, FVIIa, TGT and HPA than those with localized or advanced disease. Patients with VTE had higher baseline levels of DDi, TGT, shorter PPL and lower levels of HPA as compared to those without. Patients who died within 3-months had higher baseline levels of DDi and lower HPA levels as compared to those who were alive.

Conclusion: Increased PPL, TF pathway up regulation, DDi and HPA increase is a universal phenomenon in LA. CTx has an impact on TGT and HPA levels. Baseline values of TGT, PPL, HPA, DDi were related with mortality and thrombosis. The incorporation of HB in VTE-RAMs might improve their predictive value. This concept is being studied on an ongoing trial.

Disclosure of Interest: None declared.

PO103-TUE

Unsuspected pulmonary embolism in cancer patients: an international, ongoing, prospective, observational study

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Background: Unsuspected pulmonary embolism (UPE) is diagnosed in 1 to 5% of cancer patients undergoing routine computed tomography scans. Guidelines suggest that UPE should receive similar treatment as

for symptomatic PE. However, direct evidence is scarce and whether selected subgroups such as those with isolated (sub)segmental UPE may be treated more conservatively remains unknown.

Aims: We aim to evaluate the current treatment approaches for UPE and assess their efficacy and safety.

Methods: This is an ongoing, observational, international, multicenter study of consecutive cancer patients with UPE followed up to 12 months for the occurrence of recurrent venous thromboembolism (VTE), bleeding, and mortality. A sample size of 610 patients was calculated based on an assumed overall recurrent VTE rate of 13% and an odds of 3:1 of (sub)segmental UPE vs. central UPE. The cumulative incidence of recurrent VTE was adjusted for death as a competing event.

Results: From April 2012 to January 2015 a total of 212 patients were enrolled, of whom 119 (56.1%) had lobar or central PE. 202 patients (95.3%) received anticoagulant therapy, most often low molecular weight heparin (80.2% of cases). The median follow-up duration was 182 days (Interquartile Range 107–293). The overall cumulative incidence of recurrent VTE and all-cause mortality after 1 year was 5.7% and 37.3%, respectively. When comparing patients with central and (sub)segmental UPE, the incidence of mortality was 42.7% and 30.5% ($P = 0.9$), and the cumulative incidence of recurrent VTE 8.2% vs. 3.4% ($P = 0.32$) respectively. Bleeding occurred in 9.4% of all patients; these events comprise major, clinically relevant non major, and minor bleeding.

Conclusion: The interim results from this registry indicate that robust data are collected about the natural history of UPE, on which future (comparative) studies can build.

Disclosure of Interest: None declared.

PO104-TUE

Risk of subsequent cancer after a venous thromboembolism – the Scandinavian Thrombosis and Cancer (STAC) Study

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Background: Patients with venous thromboembolism (VTE) have an increased risk of subsequent cancer. Subjects with unprovoked VTE are considered at highest risk, but the benefit of examination for occult cancer is debated.

Aims: To study the association between VTE and subsequent cancer in the Scandinavian Thrombosis and Cancer (STAC) Study with individual data merged from three prospective population-based cohorts in Norway and Denmark.

Methods: Overall, 144912 subjects aged 19–101 years without previous VTE or cancer were enrolled, and baseline information (1993–97) included physical examination, self-administered questionnaires and blood samples. Validated VTE events and cancer diagnoses were registered up to 2007–2012 with a median follow-up of 13.6 years. We computed crude incidence rates (IRs) of cancer per 1000 person-years (PY) and multivariable adjusted hazard ratios (HRs) for cancer with 95% confidence intervals (CIs) in subjects with and without VTE. Regional committees of research ethics approved the study, and participants gave their informed written consent.

Results: There were 1884 subjects with VTE and 17651 with cancer during follow-up. The IR of cancer in subjects without VTE was 9.5 per 1000 PY, whereas the IR of cancer during the first year after VTE was 60.6 per 1000 PY. A VTE event was associated with a 4.0-fold (95% CI 3.25–5.02) higher risk of cancer during the first year after VTE, and a 1.3-fold (95% CI 1.12–1.53) higher risk for subsequent years. The risk was most pronounced for kidney, ovarian, lymphatic, pancreatic, stomach and lung cancer. Only minor differences were found in cancer risk between subjects with unprovoked (HR 4.47, 95% CI 3.43–5.83) and provoked VTE (HR 3.51, 95% CI 2.39–5.17) the first year of follow-up.

Conclusion: The risk of subsequent cancer did not differ between subjects with unprovoked and provoked VTE. Our findings suggest that the benefits of examination for occult cancer should be studied in subjects with both unprovoked and provoked VTE.

Disclosure of Interest: None declared.

PO105-TUE

Management of cancer associated thrombosis in France: a national survey in vascular disease and supportive care specialists

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Background: Low molecular weight heparins (LMWHs) are recommended by international guidelines for 3–6 months in patients with cancer associated thrombosis (CAT). However surveys report a low implementation of guidelines in usual care (around 50%)

Aims: To assess the knowledge of guidelines by vascular specialists and oncologists in France. To try to explain the lack of implementation of guidelines

Methods: The French Society for Vascular Medicine (SFMV) and Association Francophone pour les Soins Oncologiques de Support (AFSOS) contacted their members. An electronic survey tool was used to disseminate the same survey about CAT treatment using clinical situations: patient with lung cancer and symptomatic deep venous thrombosis (DVT) or pulmonary embolism (PE) or incidental PE. Answers were compared to the reference defined by a multidisciplinary panel before the survey

Results: 401 specialists completed the survey. Respondent's specialties included oncology (12%), vascular medicine (68%), hematology (2%), internal medicine (3%), pneumology (2%), others (15%). Full dose LMWHs were indicated as first choice by more than 90% for the long-

term treatment of symptomatic DVT and PE. They opted for a treatment duration of 3 months (20% for DVT, 5% for PE), 6 months (70% for DVT, 67% for PE), or 12 months (9% for DVT, 27% for PE). In case of active cancer, after 12 months, respondents chose full dose LMWHs (51% for DVT, 50% for PE), prophylactic dose LMWHs (19% for DVT, 16% for PE), VKA (13% for DVT, 14% for PE). In case of incidental recent proximal PE, 91% of respondents chose full-dose LMWHs while in front of a proximal old PE, 22% gave full dose LMWHs, 31% prophylactic LMWHs and 41% no treatment.

Conclusion: These findings illustrate that vascular specialists and oncologists implicated in supportive care are aware of recommendations for CAT treatment, resulting from the actions led by both societies. It is important to provide information about guidelines to other specialists to improve the implementation of recommendations.

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PO106-TUE

Venous thromboembolism after surgical treatment of skeletal metastases – an underdiagnosed complication

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Background: Venous thromboembolism (VTE) is a severe complication after major orthopedic surgery and especially in association with cancer. However, limited attention is paid on this topic both in clinical management and studies concerning survival and complications after surgery of skeletal metastases and fractures they induce.

Aims: We conducted this study to evaluate the incidence of VTE and its clinical impact in association with cancer surgery targeted to the management of fractures due to skeletal metastases.

Methods: Data were collected retrospectively from the medical files of Tampere University Hospital. Surgery was performed between April 1999 and July 2014. The incidence of pulmonary embolism (PE) and deep vein thrombosis and their risk factors were assessed using binary logistic regression analysis. A Kaplan-Meier analysis and a Cox-Regression analysis were utilized to evaluate variables affecting survival.

Results: Study included 343 non-spinal skeletal metastases consecutively operated in 306 patients. The rate of VTE events was as high as 11.4% and appeared on average within 70 days of surgery. Intraoperative oxygen saturation drop and pulmonary metastases were risk factors for early PE. One-year survival was significantly reduced among these PE patients, being only 18%, vs. 42% among those patients who did not experience VTE ($P < 0.01$). Other indicators of decreased survival were leukocytosis ($> 9 \times 10^9 \text{ L}^{-1}$) and anemia (hemoglobin $< 100 \text{ g dL}^{-1}$). 83% received low molecular weight heparin as thromboprophylaxis, but its duration was variable.

Conclusion: Relationship between fracture of bone in association with skeletal metastases and thromboembolic events needs both clinical and research attention. Whether survival for the patients suffering from fractures of skeletal metastases can be improved by better targeted thromboprophylactic means should be further studied.

Disclosure of Interest: None declared.

PO107-TUE

Major and non-major clinically relevant bleeding in patients hospitalized in palliative care units: results of a French multicentre observational study

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Background: While there is an increasing prescription of thromboprophylaxis in Palliative Care (PC) inpatients, the true incidence of both bleeding and venous thromboembolism (VTE) in such patients is unknown.

Aims: To evaluate in PC inpatients the incidence and the risk factors of Clinically Relevant Bleeding (CRB) and of venous thromboembolism.

Methods: A French multicentre prospective cohort study (RHESO, NCT01056978) was conducted in 22 PC Units. To be included, patients should not receive therapeutic anticoagulation on admission and their life expectancy was expected to exceed 48 h. The primary outcome was the incidence at 3 months of major and/or non-major CRB. All events were centrally adjudicated by an independent committee. The cumulative incidence of events was estimated accounting for the presence of competing risk events. Multivariate analysis was performed to determine risk factors for CRB using Cox model accounting for the presence of competing risk events.

Results: From June 2010 to October 2011, 1199 patients were enrolled in the RHESO study. The most common medical reason of palliative care was metastatic cancer (77.7%). At three months, the mortality rate was 91.3% and 116 patients presented a CRB (9.7%–95% CI: 8.1–11.6), given a cumulative incidence of CRB of 9.8% (95% CI: 8.3–11.6). The median delay of CRB was 7.5 days (IQR: 3.5–21.5). The most common site of major bleeding was digestive tract. Of the 32 major bleedings, 23 contributed to death. In multivariate analysis, cancer [HR = 4.09 (95% CI: 1.30–12.9), $P = 0.02$], previous surgery within the four weeks before PC Units admission [HR = 0.25 (95% CI: 0.06–1.02), $P = 0.053$] and treatment with antiplatelet agents [HR = 1.70 (95% CI: 1.17–2.48), $P = 0.005$] were found as independent factors associated with CRB. At three months, the cumulative incidence of VTE was 0.3% (95% CI: 0.1–0.8).

Conclusion: This unexpected high bleeding risk must be taken into account in the management of palliative care inpatients.

Disclosure of Interest: None declared.

PO108-TUE

Healthcare related cost of thrombotic events in elderly patients: a retrospective database analysisOladapo A¹, Xiong Y¹, Novack A² and Epstein J¹¹Medical Outcomes Research & Economics; ²Global Medical Affairs, Baxter Healthcare Corporation, Westlake Village, USA

Background: Elderly patients have a high risk for developing thrombotic events (TEEs) given their advanced age and higher risk of developing multiple comorbidities including bleeding conditions such as acquired hemophilia A.

Aims: To estimate the annual healthcare cost associated with the management of TEEs in elderly patients.

Methods: A retrospective analysis of MarketScan database (medical/prescription claims) over an 8-year period (January 2006 to March 2014) was conducted. Subjects were included if they: (i) were ≥ 65 years; (ii) had an inpatient primary or secondary diagnosis (based on ICD-9-CM codes) for a TEE; (iii) were continuously enrolled for ≥ 12 months prior to and ≥ 12 months following the index date; and (iv) had no diagnosis for a TEE in the 12-month pre-index period. The index date was the date of the first inpatient diagnosis of a TEE. Healthcare cost associated with treating TEEs in the first year of treatment (12-months post-index) was estimated and adjusted to 2013 U.S. dollars.

Results: A total of 145,067 subjects were identified of which 51.4% were females. The mean ($\pm$ SD) age at index was 78 ($\pm$ 8) years. The number of events per patient-year was 1.3. Ischemic related TEEs were the most common (0.4 events per patient-year; 31% of all TEEs). The mean annual healthcare cost associated with treatment of TEEs was \$23,771 (standard error (SE) = $\pm$ \$112). In-patient related cost accounted for 93.4% of the total annual healthcare cost. When in-patient related cost was stratified by type of TEE, mean annual cost ranged from \$15,206 (SE = $\pm$ \$131) to \$59,103 (SE = $\pm$ \$4853). Disseminated intravascular coagulation (\$59,103; SE = $\pm$ \$4853), cardiac arrest (\$44,625; SE = $\pm$ \$1793) and hepatic infarction (\$35,724; SE = $\pm$ \$7174) ranked as the top 3 most expensive TEEs.

Conclusion: Healthcare related cost associated with treatment of TEEs in the elderly may be significant and varies depending on the type of TEE.

Disclosure of Interest: None declared.

PO109-TUE

The utility of PEmb-QoL and VEINES-QoL/Sym questionnaires in evaluating quality of life in the cancer associated thrombosis patientProut H¹, Nelson A¹ and Noble S²¹Marie Curie Palliative Care Research Group; ²Oncology, Cardiff University, Cardiff, UK

Background: Whilst the clinical management of cancer associated thrombosis (CAT) is clearly established, the impact of CAT on the patient's quality of life (QoL) is yet to be formally evaluated. The majority of research looking at QoL and venous thromboembolism (VTE) has been conducted in non cancer patients. Likewise, the current validated QoL questionnaires were designed without specific consideration of CAT or the overall cancer journey. We therefore to ascertain if current validated VTE QoL tools are applicable to CAT?

Aims: A main objective of the study was to ascertain if current validated VTE QoL tools are of meaning and relevance for CAT patients?

Methods: Following a semi-structured interview regarding their experiences of CAT, twenty patients completed the PEmb-QoL or VEINES-QoL/Sym questionnaire(s) using cognitive interviewing techniques. The questionnaires were to be analysed using both deductive and inductive methods

Results: Patients found the tools easy to understand and answer, thus negating the need for cognitive interview techniques. Thematic analysis suggested, however, that patients felt these tools were of limited utility in the cancer context since they related predominantly to the impact of VTE on a background of previous good health. Patients found it difficult to identify which affected QoL domains were due to their cancer and which their VTE. Patients viewed themselves primarily as cancer patients and therefore the QoL tools did not adequately address the daily issues that affected them most.

Conclusion: Just as the treatment of CAT differs fundamentally from those with VTE and no cancer, so too will the impact of the VTE. Consequently the current available QoL tools, developed in the non cancer population have limited utility in evaluating QoL in CAT patients. Cancer specific QoL tools are therefore needed to holistically evaluate the impact of VTE in the cancer setting.

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PO110-TUE

Patients' preferences for the treatment of cancer associated thrombosisNoble S¹, Maraveyas A², Matzdorff A³, Holm MV⁴ and Pisa G⁵¹Oncology, Cardiff University, Cardiff; ²Oncology, Hull York Medical School, York, UK; ³Oncology, Caritasclinic, Saarbruecken, Germany; ⁴Leo Pharma, Ballerup, Denmark; ⁵Kantar Health, Munich, Germany

Background: Clinical decision-making is based on more than evidence alone. Although clinical guidelines recommend Low Molecular Weight Heparin (LMWH) for the treatment of cancer-associated thrombosis (CAT) it has been suggested patients may favour oral agents such as warfarin or a Non-vitamin K antagonist Oral Anticoagulants (NOACs) on the grounds of convenience and quality of life. To date, there has been no evaluation of patient preferences and the importance CAT patients place on different drug attributes.

Aims: To evaluate what features are most important to CAT patients regarding their choice of anticoagulant.

Methods: Following an initial qualitative module, a choice-based experiment was undertaken with 100 CAT patients from Germany ($n = 50$) and the UK ($n = 50$) to identify which attributes were most important when choosing an anticoagulant. Using conjoint methodology, the relative importance of drug attributes was assessed individually, resulting in the identification of the most valuable attributes of an anticoagulant.

Results: 61% of the patients in the sample had deep vein thrombosis (DVT), 35% had pulmonary embolism (PE), and 4% had both. As for the current VTE therapy, 70% of the patients were treated with anticoagulation by injection (LMWH) and 30% with an oral drug (20% NOAC, 10% warfarin). Patients most valued an anticoagulant with minimal interference with their cancer treatment (39%), low thrombosis recurrence rate (24%), and low risk of a major bleed (19%). Preference for oral administration over injection had moderate importance (13%), whereas risk of minor bleeds, frequency of administration and monitoring requirements were of minor relevance.

Conclusion: Patients primarily want an anticoagulant that does not interfere with their cancer treatment, suggesting the primacy of the cancer disease over VTE for these patients. They also value efficacy and safety above convenience of route of administration

Disclosure of Interest: S. Noble Grant/Research Support from: LEO Pharma, Pfizer, Bristol Meyers Squibb, Boehringer Ingelheim, Consultant for: Pfizer, Leo Pharma, Bristol Meyers Squibb, Sanofi Aventis, A. Maraveyas Grant/Research Support from: LEO Pharma, Pfizer, Consultant for: Pfizer, Leo Pharma, Sanofi Aventis, A. Matzdorff Grant/Research Support from: LEO Pharma, Merck Serono, Roche,

Consultant for: AMGEN, GlaxoSmithKline, Baxter, LEO Pharma, Boehringer Ingelheim, M. Vindt Holm Employee of: Leo Pharma, G. Pisa Employee of: Leo Pharma

PO111-TUE

Age should not be considered a risk factor for cancer-related venous thromboembolism: results from the Scandinavian thrombosis and cancer study

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Background: Venous thromboembolism (VTE) is common in cancer patients and targeted prevention is warranted. It is not known whether age should be considered a risk factor for VTE in cancer patients.

Aims: To investigate the association between age and VTE risk during the first year after cancer diagnosis in a population-based cohort.

Methods: Individual data from the Tromsø Study, the HUNT2 Study and the Diet, Cancer and Health Study were merged and a total of 137,273 initially cancer-free subjects were followed from inclusion (1993–97) until 2010. Cancer diagnoses were provided by the national cancer registries, and VTE events were validated in all three cohorts. Regional committees of research ethics approved the study, and participants gave their informed written consent.

Results: A total of 386 VTE events were observed among 13,024 subjects diagnosed with cancer during follow-up. The incidence rates (IR) were between 16–21 per 1000 person-years (PY) in all ten-year categories up to 80 years, but was slightly higher in those ≥ 80 (IR 31 per 1000 PY). In the analyses on cancer types, only patients with prostate cancer had a clearly higher risk of VTE at high age. Here, IRs ranged between 0–5 per 1000 PY for the age-groups below 80 years, but increased to 36 per 1000 PY in patients above 80 years. For other subtypes of cancer (i.e. lung-, brain-, colorectal- and ovarian cancer) there was no trend across age-categories, and for pancreatic cancer there was even an inverse relationship between VTE and advanced age.

Conclusion: We found a slightly higher risk of cancer-related VTE in patients ≥ 80 years than in younger patients. However, this finding was inconsistent across cancer sites, and was mainly due to a higher risk of VTE in prostate-cancer patients ≥ 80 years. Thus, we suggest that age should neither be considered a risk factor for cancer-related VTE nor included in risk prediction models

Disclosure of Interest: None declared.

PO112-TUE

Cancer-related venous thromboembolism in the general population: results from the Scandinavian Thrombosis and Cancer (STAC) study

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Background: Cancer patients are reported to have a 4–7 fold increased risk of VTE. However, these estimates are mainly retrieved from registry studies without validated VTE events or confounder information, and are based on long-term follow-up.

Aims: To study the association between cancer and risk of VTE focusing on duration of follow-up after cancer diagnosis in a population-based cohort.

Methods: Participants were recruited from the Tromsø IV Study, the HUNT2 Study and the Diet, Cancer and Health Study, and 137,273 subjects were followed from inclusion (1993–97) to 2010. Cancer diagnoses were obtained from the national cancer registries, and VTE events were validated in all three cohorts. Regional ethical committees approved the study, and participants gave their informed written consent.

Results: Overall, 3% of the cancer patients were diagnosed with VTE over the study period and cancer explained 20% of all VTEs. The VTE rates were 12 per 1000 person-years (PY) in the last 6 months before cancer diagnosis, 29 per 1000 PY in the 0–6 month period, and 8 per 1000 PY in the 6–12 month period after diagnosis. The 1-year hazard ratio (HR) for VTE by cancer was 12-fold increased (HR 11.7; 95% CI 10.1–13.6), and declined to 5.8 (95% CI 5.1–6.6) when follow-up was extended to the first 5 years. The 1-year HR was highest for pancreatic (HR 39.3; 95% CI 22.2–69.4), lung (HR 30.5; 95% CI 22.8–40.9) and brain cancer (HR 25.9; 95% CI 14.3–47.0), and was lowest for breast- and prostate cancer (HR 5.5; 95% CI 3.2–9.7 and HR 2.7; 95% CI 1.5–4.7, respectively). At the population level, lung-, colorectal- and prostate cancer explained the largest proportion of VTEs.

Conclusion: We found that the VTE risk in cancer patients is strongly related to time of cancer diagnosis and that the majority of VTEs occur within a year. This suggests that time-restricted estimates should be used in risk prediction models.

Disclosure of Interest: None declared.

PO113-TUE

Incidence, risk factors, and outcome of early hemostatic complications in patients after allogeneic hematopoietic stem cell transplantation: a retrospective multicenter study of 551 patients

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Background: Hemostatic disorders are common and potentially fatal complications in patients undergoing allogeneic hematopoietic stem-cell transplantation. However, Limited data exists on early diagnosis and prevention of these complications.

Aims: To investigate incidence, risk factors, and outcome of early hemostatic complications in patients after allogeneic hematopoietic stem cell transplantation.

Methods: 551 allogeneic transplantation recipients were enrolled to investigate the incidence, risk factors, and outcome of thrombotic or bleeding complications in the first 100 days after transplantation. Of all the patients, 261 cases (47.4%) developed bleeding events, the cumulative incidence of minor, moderate, and severe bleeding was 28.9%, 14.9% and 3.8%, respectively.

Results: The incidence of thrombotic complications was 4.5%, consisting of 15 cases of veno-occlusive disease, 7 thrombotic microangiopathy, 2 pulmonary embolism, and 1 deep vein thrombosis. Risk factor analysis demonstrated that veno-occlusive disease, II-IV acute graft-vs.-host disease (GVHD) and cord blood transplantation were independent predictors for bleeding complications in multivariate analysis. Meanwhile, mismatched donor, polyomavirus BK infection, cytomegalovirus infection and II-IV acute GVHD were potential risk factors for late-onset hemorrhagic cystitis, while total body irradiation conditioning regimen and high-risk disease status prior to transplantation were significantly associated with the occurrence of thrombotic disorders. Severe hemorrhage and early-onset of thrombotic disorders independently increased the mortality of allogeneic transplantation recipients.

Conclusion: Our study demonstrated that hemostatic complications following transplantation have much high mortality. Therefore, early diagnosis and therapeutic intervention of hemostatic complications are crucial to improve the outcome of allogeneic hematopoietic stem cell transplantation recipients.

Disclosure of Interest: None declared.

PO114-TUE

Detection of hypercoagulability in hepatocellular carcinoma (HCC)

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Background: Patients with liver cirrhosis are generally considered to have an increased bleeding risk but are now thought to also have increased thrombotic risk.

Aims: To evaluate global hypercoagulability in cirrhotic patients with and without hepatocellular carcinoma (HCC) using factor FVIIa-anti-thrombin complex (FVIIa-AT), a marker for tissue factor expression/activity, procoagulant microparticles activity (PPL) as a marker of cellular blood components activation and activated TAFI as a measure of fibrinolysis activation.

Methods: The results in cirrhosis patients with HCC ($n = 33$) were compared with those of a non-HCC group ($n = 33$) with liver cirrhosis

and a group of healthy ($n = 33$) volunteers. Informed consent was obtained from all subjects.

The FVIIa-AT, FVIIa activity, AT, procoagulant activity (PPL) and activated TAFI (TAFIa/ai) was measured in all samples.

Results: The levels of FVIIa-AT were similar in the HCC and non-HCC group (60 ± 14 vs. 61 ± 24 pm). FVIIa-AT in HCC patients was significantly lower than in healthy subjects (108 ± 18 pm, $P < 0.05$). The levels of FVIIa were significantly lower in HCC and non-HCC (61 ± 20 and 52 ± 21 mU mL⁻¹, respectively) than in the group of healthy controls (94 ± 22 mU mL⁻¹, $P < 0.05$). AT was significantly lower in both HCC and non-HCC (61 ± 20 and $61 \pm 26\%$) compared with controls ($98 \pm 15\%$, $P < 0.005$). PPL activity in HCC was significantly lower in the healthy subjects (61 ± 10 v 81 ± 8 s, $P < 0.01$) and similar to those found in non-HCC (61 ± 24 s). A shortening of the PPL clotting time reflects increased procoagulant activity. No increase in TAFI activations was seen.

Conclusion: The coagulation pathway is down regulated in cirrhotic patients, with and without HCC while the cellular/microparticle derived procoagulant activity is up regulated. If this *in vitro* hypercoagulability due to increased PPL activity reflects the *in vivo* state remains to be established. Further studies are required to see if this has clinical relevance.

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PO115-TUE

Role of arterial cardiovascular risk factors in the pathogenesis of cancer related VTE

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Background: Risk scores were developed to identify cancer outpatients at high risk for venous thromboembolic events (VTE) but current evidences are insufficient to recommend a routine use of antithrombotic prophylaxis even in high risk patients. Several studies suggested that arterial cardiovascular risk factors may be involved also in the pathogenesis of VTE. However, to date, no study has properly assessed if these risk factors may have a role also in the pathogenesis of cancer associated VTE.

Aims: To assess the role of cardiovascular risk factors including abdominal obesity, diabetes mellitus (DM), hypertension, smoking and dyslipidemia in the pathogenesis of VTE in a large cohort of consecutive cancer patients

Methods: Patients with a new diagnosis of cancer evaluated at the ambulatory of the Oncology Clinic of Varese, Italy were included. VTE incidence was recorded during a 6-month follow-up period. Univariate and subsequent multivariate analyses were performed to identify independent VTE predictors.

Results: 1309 patients (mean age 62.3 years, 63% males) were included, 68.5% were on chemotherapy. Complete follow up was available for the whole population. At the end of follow up, 66 patients (5.04%) had a VTE. At the univariate analysis smoking (OR 2.45, 95% CI 1.31, 4.59) and hypertension (OR 1.63, 95% CI 1.00, 2.66) were significantly associated with VTE risk whereas DM, obesity and dyslipidemia were not. Furthermore, age, previous VTE, very high risk cancer type (stomach and pancreas) and presence of metastasis were significantly or marginally significant associated with VTE ($P < 0.10$). At the multivariate analysis only previous VTE and very high risk cancer type remained significantly associated with VTE (OR 13.77, 95% CI 6.94, 27.34 and 2.31, 95% CI 1.29, 4.13 respectively)

Conclusion: The role of traditional cardiovascular risk factors in the pathogenesis of cancer related VTE appeared limited. Other studies are necessary to confirm our preliminary findings.

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PO116-TUE

Factors influencing the use of thromboprophylaxis in ambulatory cancer patients in the real world clinical practice

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Background: Some studies described the factors that influence thromboprophylaxis in hospitalized cancer patients, but there are few data in ambulatory patients. Current guidelines do not recommend routine thromboprophylaxis in this setting. However, the benefit/risk ratio may favor it in a subgroup of high-risk patients.

Aims: To prospectively evaluate factors influencing thromboprophylaxis use in cancer patients receiving ambulatory chemotherapy in the real world clinical practice at an academic center.

Methods: Consecutive adult patients with an active malignancy receiving ambulatory chemotherapy were included. Those under therapeutic anticoagulation were excluded. Risk factors for VTE were collected by using a software linked to our Clinical Records System. Patients were followed for 90 days. LMWH use, incidence of VTE and major bleeding during follow-up were obtained through medical records review or by telephone interview.

Results: Between November 2008 and December 2010, 1108 patients (mean age 58 ± 12 years; 49% male) were included (Khorana's score: 46% low, 47% intermediate, 7% high). LMWH prophylaxis (median duration 24 days) was given at any time to 264 patients (23.8%). After multivariable logistic regression, the strongest predictors of LMWH prescription were intercurrent hospitalization and previous VTE ($P < 0.001$). Thromboprophylaxis was also more frequent in male patients ($P = 0.04$) or those receiving first-line chemotherapy ($P = 0.007$). Recent surgery was negatively associated with the use of LMWH ($P = 0.02$).

During follow-up, 58 VTE events were observed, 10 of them despite LMWH use. The only independent predictor of VTE was the malignancy origin (OR 3.48, 95%CI 1.43–8.46 and OR 2.52, 95%CI 1.19–5.28 for pancreas and lung cancer, respectively). 9 major bleeding events were recorded, 4 of them associated with LMWH prophylaxis.

Conclusion: In the real world clinical practice, the use of LMWH prophylaxis in ambulatory cancer patients is limited. The influence of concomitant risk factors for VTE is highly variable.

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PO117-TUE

Residual vein thrombosis to assess the duration of low molecular weight heparin (the extended cancer-DACUS)

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Background: The optimal duration of Low Molecular Weight Heparin (LMWH) after cancer associated deep vein thrombosis (DVT) is unknown; current guidelines suggest to prolong anticoagulation until cancer is active.

Aims: We prospectively evaluated a RVT-based management of patients with cancer-associated DVT; LMWH treatment was extended up to 2 years in patients at high-risk of recurrent DVT for persistent RVT

Methods: Patients were included at the time of a first diagnosis of cancer-associated DVT of the lower limbs. All received LMWH at therapeutic dosage for the first month then reduced at 75% for the following months. After 6 months, RVT was evaluated: patients without RVT (suspended treatment, while those with RVT continued LMWH for up to 2 years. Recurrent thrombosis and/or bleeding events were recorded during treatment and one year after LMWH withdrawal. Baseline differences between groups were assessed by the chi-square test (Yates' correction) for categorical variables and ANOVA test or Kruskal-Wallis test for parametric and nonparametric analyses. Relative risks (RRs) and 95% confidence intervals (CIs) were evaluated

Results: Overall, 211 cancer patients were enrolled; RVT was detected in 129 patients (61.1%). Recurrent VTE occurred in 19 (14.7%); 4 episodes (3.1%) occurred while on heparin. Among patients without RVT (82), 3 (3.6%) developed recurrent VTE (after LMWH therapy). Adjusted HR for RVT vs. no-RVT group was 5.8 (95% CI, 1.9 to 19.2; $P = 0.0003$). Three major bleeding events occurred in RVT group and one in no-RVT group (during LMWH administration). The HR for major bleeding (RVT vs. no-RVT group) was 2.58 (95% CI, 0.66 to 12.43; $p = 0.103$). Overall, 44 patients (20.8%) died during follow-up for cancer progression.

Conclusion: In patients without RVT, a short period of treatment with a LMWH is sufficient; in those with persistent RVT, treatment extended to 2 years substantially reduces, but does not eliminate, the risk of recurrent thrombosis. However, when still on LMWH, the risk for recurrent VTE is low

Disclosure of Interest: None declared.

PO118-TUE

Between a rock and a hard place: conundrums in the management of cerebral sinovenous thrombosis (CSVT) in children with acute lymphoblastic leukemia (ALL)/lymphoma

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Background: Anticoagulation therapy (ACT) for CSVT in patients (pts) receiving antileukemic therapy is challenging, especially in presence of a bleed or at risk for coagulopathy.

Aims: To describe ACT and outcomes of CSVT in pts receiving asparaginase-(ASP)-based chemotherapy.

Methods: Diagnosis, therapy, and outcome data of pts consecutively diagnosed with CSVT at the two participating centers between 2000–2014 were collected. ACT and CSVT imaging protocols were in place in both centers. Ethics approval was obtained with consent waiver.

Results: Thirty-three pts [mean (range) age: 9.1 (2–18) years, 20 boys] had CSVT; 7/33 (21%) presented with intracerebral hemorrhage. Thirty-two pts (96%) received ACT [unfractionated/low molecular weight heparin (LMWH) (16), LMWH alone (16)] at a mean of 1.3 (0–27) days after CSVT diagnosis. Mean ACT duration was 7 (1–18) months. ACT associated bleeding occurred in 2 pts; 1 major (bleed at diagnosis) and 1 minor (no prior bleed). CSVT progressed in 7 (21%) pts; 5 in first 2 weeks. Two of 7 pts with initial bleed had progression compared to 5 of 25 without bleed ($P = 0.63$). Progression was associated with chemotherapy phase (ALL Induction 37% vs. post-induction 6%, $P = 0.04$). Radiological complete/near complete resolution was attained in 22/32 (69%) pts [mean time: 3.7 (1–11) months]. No CSVT-related deaths occurred and 5 (15%) pts had neurologic sequelae. ASP data were missing for 3 pts; 1 had completed ASP prior to CSVT. Of remaining, 24/29 (83%) had ASP re-instated with LMWH without CSVT progression and 5 discontinued (2 CSVT-related, 3 other).

Conclusion: In children receiving myelosuppressive therapy, ACT-associated major bleeding (3%) is comparable to that reported in literature for CSVT in children, and ACT resulted in resolution of CSVT in 69% pts. CSVT diagnosis during induction therapy is associated with higher risk of progression indicating the need for optimization of ACT during this phase of antileukemic therapy. ASP therapy can be safely re-instated with concurrent ACT.

Disclosure of Interest: None declared.

PO119-TUE

A prospective study on the incidence of postoperative venous thromboembolism in Korean colorectal cancer patients

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Background: To prevent postoperative venous thromboembolism (VTE), perioperative pharmacologic thromboprophylaxis is recommended for all surgical cancer patients in Western guidelines. However, Asian patients are known to have lower risk of VTE than Western patients.

Aims: This prospective study was conducted to investigate incidence and risk factors of postoperative VTE in Korean patients with colorectal cancer (CRC) receiving major abdominal surgery.

Methods: This study was composed of two cohorts. All patients did not receive perioperative pharmacologic thromboprophylaxis. In cohort A, patients were routinely screened for the development of VTE with lower-extremity Doppler ultrasonography (LE-DUS) on postoperative days 5–12; thereafter, LE-DUS or other studies were conducted only when symptoms/signs suspicious for VTE developed. In cohort B, routine LE-DUS was not conducted; image studies were carried out only if there was a clinical suspicion of VTE development. The primary endpoint was the incidence of postoperative VTE in 4 weeks after surgery in cohort A. Risk factors for postoperative VTE were also analyzed.

Results: Between September 2011 and March 2014, 548 patients (400 in cohort A and 148 in B) were enrolled at Seoul National University Bundang Hospital. In cohort A, the incidence of VTE in 4 weeks after

surgery was 3.0% ($n = 12$). Among these 12 patients, 8 patients had distal calf vein thrombosis and only one patient had symptomatic thrombosis. Age ≥ 70 years [odds ratio (OR) 5.61; $P = 0.048$], two or more comorbidities (OR 13.42; $P = 0.025$) and leukocytosis ($> 10,000 \mu\text{L}^{-1}$; OR 17.43; $P = 0.002$) were independent risk factors for postoperative VTE development. In cohort B, only one symptomatic postoperative VTE case (0.7%) was observed.

Conclusion: Postoperative VTE in Korean CRC patients was only 3.0%. In Asian cancer patients, perioperative pharmacologic thromboprophylaxis needs to be based on a risk-based approach rather than routine basis.

Disclosure of Interest: None declared.

PO120-TUE

Thromboembolic events in children with cancer in Srinagarind hospital

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Background: The previous reported incidence of symptomatic venous thromboembolism (VTE) in children with cancer ranges between 2.1 and 16%. The incidence rate of VTE in Thai children was 3.9/10,000 hospital per year.

Aims: To describe clinical manifestations, precipitating factors, treatment and outcome in children with cancer who developed VTE

Methods: 611 children diagnosed with cancer from 2009 to 2014 were retrospectively reviewed. Only children with thrombosis are included.

Results: The incidence of thrombosis is 13/611 (2.12%) [7 females and 6 males (age 22 month to 16 years)]. Cancer types include B-cell acute lymphoblastic leukemia (ALL) (5), T-cell ALL (1), Lymphoma (1), Wilms tumor (2), endodermal sinus tumor (1), rhabdomyosarcoma (1), hepatoblastoma (1), craniopharyngioma (1). Precipitating factors for thrombosis are from L-asparaginase (3), central line catheter (CVL = 3), tumor compression (5), sinusitis (1) and unknown (1). The presenting symptoms depending on location of thrombosis include hemiparesis (1), seizure (3), ophthalmoplegia (1), asymptomatic (4), leg swelling (3), dyspnea (1). Locations of thrombosis are as follows; arterial stroke = 3 (right MCA, unilateral Pon, and right cerebellar hemisphere), cavernous sinus (1), sagittal sinus (1), inferior vena cava (IVC) (2), deep venous of leg (4), pulmonary embolism (1), renal vein (2), left hepatic and middle hepatic vein (1). Treatment for thrombosis are given as following; complete course of LMWH = 7, no anticoagulation = 2, standard heparin = 1, incomplete course of LMWH = 2, and ongoing LMWH = 1. The outcome of thrombosis treatment is death from pontine stroke (1), and from diseases (3), complete resolution of thrombosis and alive (8), and ongoing treatment (1).

Conclusion: The incidence of thrombosis is similar to previous studies. Risk factors of thrombosis are from tumor itself, treatment and CVL. Anticoagulation is more precise to completely resolve the thrombosis. Awareness of VTE is influenced to the outcome of thrombosis and cancer.

Disclosure of Interest: None declared.

PO121-TUE

Thromboembolic complications in patients with lymphoma

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Background: An increased risk for thromboembolism in cancer patients has been observed in patients with solid tumours, whereas little data exist on malignant lymphoma. In few published studies the rate of thrombotic complications in lymphoproliferative disease is highly variable

Aims: Aim of our study was to determine incidence of thromboembolic events (TE) in patients with non Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL) and chronic lymphocytic leukemia (CLL) who were treated in our institution and as well as to assess utility of Khorana and Padua scores in analyzed group of patients.

Methods: We reviewed all medical records of patients with NHL, HL and CLL diagnosed according to the World Health Organisation classification and treated at our institution between January 2006 and Decembre 2014.

Results: A total of 1596 patients, with malignant lymphoma were eligible for analysis. Out of our lymphoma patients, 71 (4.4%) had one TE. A total of 36 patients were men and 35 women with mean age of 64.5 years. Thromboembolic events included deep vein thrombosis ($n = 29$), pulmonary embolism ($n = 6$), vena jugularis interna thrombosis ($n = 9$), CNS thrombosis ($n = 6$), arterial thrombosis ($n = 2$) and other ($n = 19$). A total of 49 TE (72%) occurred during treatment and during 3 months after completion of therapy, whereas 22 TE (28%) were diagnosed prior to therapy. Most of patients with TE had aggressive NHL ($n = 57$, 80.3%). Only 12 patients (16.9%) were classified as high risk patients for TE development with Chorana score while using Padua score 29 patients (40.8%) were assessed with increased risk for TE.

Conclusion: We need a better score to help discriminate between patients with lymphoma at high and low risk of VTE. The adoption of adequate thromboprophylaxis in high-risk patients during hospitalization leads to longstanding protection against thromboembolic events with a low risk of bleeding.

Disclosure of Interest: None declared.

PO122-TUE

Selection of anticoagulation therapy in cancer patients

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Background: Thrombophilia is a serious risk factor for cancer patient:

Aims: To determine the optimal mode of appointment of antithrombotic prophylaxis in perioperative period in cancer patients

Methods: The study involved 889 patients with gynecological cancer in the perioperative period.

Group I – LMWH for 10 days before surgery 0.3 mL, cessation of therapy 24 h before surgery, then 0.3 mL for 10 days postoperatively – 213 patients

Group II – LMWH 24 h before surgery, then 0.3 mL for 10 days in postoperative period – 212.

Group III – LMWH 0.3 mL for 10 days postoperatively – 216.

Group IV – unfractionated heparin 5000 IU 3 times a day for 10 days in the postoperative period – 248.

Results: Before surgery rate of subcompensated DIC was 18.5–50%. After surgery rate of subcompensated DIC has increased significantly to 52–75%.

In group I, normal levels of DIC markers (TAT, PF4, F1 + 2) has been observed in 1–3 days.

In group II normalization of DIC markers has been observed in 3–5 days.

In group III DIC markers tended to normalize in 5–7 days.

In group IV normalization of DIC markers has been detected only on the 7th day. D-dimer in some patients remained heightened for up to 10 days. In addition, 28 patients (13.7%) formed extensive bruising in the painful injection.

Conclusion: The proposed scheme prophylaxis: LMWH 10 days before surgery and cancel 24 h prior to surgery, then 0.3 mL for 10 days in the postoperative period – virtually eliminates the risk of thrombosis and contributes normalization of DIC markers in 3 days. This scheme could be recommended for all cancer patients as a minimum program.

Disclosure of Interest: None declared.

PO123-TUE

DIC and thrombophilia during chemotherapy and its prevention approaches in ovarian cancer patients

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Background: Chemotherapy are additional risk factor for thrombosis in cancer patients.

Aims: To determine necessary range of laboratory tests for a high-grade estimation of haemostasis state in patients undergoing chemotherapy

Methods: 116 patients undergoing chemotherapy divides at random on 2 groups:

I group: 56 received biosimilar LMWH Hemapaxan 4000 IU before each chemotherapy course.

II group: 60 without any anticoagulant prophylaxis during chemotherapy.

Laboratory tests: Platelet aggregation tests, DIC and thrombophilia marker tests: D-dimer, TAT complexes, F1 + 2 prothrombin. Fibrinolytic activity tests: determine PAI level, Protein C and S levels

Results: Before operation thrombophilia and DIC was detected in 65%, subcompensated forms was in 35%.

In postoperative period in 96% were detected thrombophilia and DIC. The rate of subcompensated forms of DIC was 57%, decompensated 21%.

We have detected the sign of thrombophilia and DIC in more than 90% patient during chemotherapy. The rate of the subcompensated forms of DIC was 30%, decompensated 23%. It was observed damage of fibrinolytic activity due to iatrogenic effects of chemotherapy: reduction in proteins C and S levels, increase PAI concentration, platelets hyperaggregation in ristotetin presence.

In I group normalization of lab test results was detected during 2–3 days after chemotherapy course in comparison with II group normalisation was in 5–7 days in 22% and in 7–12 day in 58%, in 20% was not registered spontaneous normalisation

Conclusion: Due to endothelium protection activity LMWH in ovarian cancer patients during chemotherapy significantly reduce intensity of thrombophilia and DIC. 85–90% patients with cancer of female genitals required permanent preventive anticoagulant prophylaxis

Disclosure of Interest: None declared.

PO124-TUE

Mortality following venous thromboembolism: a population-based cohort study in patients with active cancerMartinez C¹, Katholing A¹, Rietbrock S¹ and Cohen A²¹*Institute for Epidemiology, Statistics and Informatics GmbH, Frankfurt, Germany;* ²*Department of Haematological Medicine, Guy's and St Thomas' Hospital, London, UK***Background:** Patients with active cancer associated venous thromboembolism (VTE) have high risk of mortality. However studies on age and sex related mortality are limited.**Aims:** N/A.**Methods:** A population based observational cohort study was undertaken to estimate the incidence of mortality in patients with a first active cancer-related VTE. The source population consisted of all patients in the UK Clinical Practice Research Datalink with additional linked information on hospitalisations and cause of death. All VTE events between 2001 and 2011 were identified using a validated algorithm. VTE events were defined as cancer-related if a clinical diagnosis or therapy for cancer was recorded in the 90 days before or after the VTE. Patients were followed up for their vital status. Mortality was estimated using survival analyses.**Results:** There were 6592 active cancer-associated VTE with a total of 7143 person-years of follow up. The mortality risk following VTE was 64.5% after 1 year, 79.0% after 3 years, 83.0% after 5 years and 88.1% after 10 years. Mortality rates were 73.9 (71.9–75.9) per 100 person years and plateaued between 30 to 69 years.

Age-specific mortality rates for those under 30 years were higher in females than males but lower than among those over 30.

Conclusion: The mortality risk following VTE was considerable in patients with active cancer.**Disclosure of Interest:** C. Martinez Grant/Research Support from: IfESI has received grants for investigator-initiated studies from Bayer Pharma AG, BMS-Pfizer and CSL Behring, Consultant for: Bayer Pharma AG and Boehringer Ingelheim, A. Katholing Grant/Research Support from: IfESI has received grants for investigator-initiated studies from Bayer Pharma AG, BMS-Pfizer and CSL Behring, S. Rietbrock Grant/Research Support from: IfESI has received grants for investigator-initiated studies from Bayer Pharma AG, BMS-Pfizer and CSL Behring, A. Cohen Consultant for: Bayer, Boehringer-Ingelheim, BMS, Daiichi, GSK, Johnson and Johnson, Mitsubishi Pharma, Pfizer, Portola, Sanofi, Schering Plough, Takeda, XO1, UK Government Health Select Committee, the Department of Health, the NHS, Lifeblood and the Coalition to Prevent Venous Thromboembolism.

PO125-TUE

Evaluation of mean platelet volume as a predictive marker for venous thromboembolism and mortality in patients treated for diffused large B-cell lymphoma

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*Department of Hematology, Poznan University of Medical Sciences, Poznań, Poland***Background:** Published data suggest that mean platelet volume (MPV) is associated with risk of venous thromboembolism (VTE) and mortality in patients with cancer.**Aims:** We evaluated the association of MPV with VTE and mortality in patients treated for newly diagnosed or relapsed/refractory diffused large B-cell lymphoma (DLBCL).**Methods:** Analyses for symptomatic VTE occurrence and all caused mortality were performed in 184 adult patients (pts) with DLBCL (median age 59, range 18–98, 55% males), 141 pts newly diagnosed and 43 with relapse/refractory lymphoma.**Results:** During the observation (median 499 days, range 2–1 855), 39 (21.2%) pts developed thrombotic events and 39 pts died (relapse/refractory disease – 10, infections – 7, toxicity – 2, cardiac arrest – 16, secondary malignancies – 2, ischemic stroke – 2 pts). In univariate analysis, including age, male gender, MPV $\leq$ 10th percentile (≤ 6.11 fl), platelet count, treatment line (relapse/refractory vs. newly diagnosed) and VTE risk (Khorana model), only MPV (OR 2.01; 95% CI 1.23–3.29, $P = 0.0053$) and treatment line were associated with VTE. In multivariate logistic regression model, MPV $\leq$ 10th percentile (OR 1.81; 95% CI 1.06–3.11, $P = 0.03$) and treatment line (OR 2.46; 95% CI 1.66–3.65, $P < 0.001$) were significantly associated with the VTE occurrence. In the Kaplan-Meier analysis, pts with MPV $>$ 10th percentile had statistically significantly longer VTE-free survival than pts with lower MPV (log rank test=2.71, $P = 0.0068$).In a multivariable Cox regression analysis, MPV $\leq$ 10th percentile (HR 2.49; 95% CI 1.18–5.21, $P = 0.016$), male gender (HR 4.31; 95% CI 1.89–9.82, $P = 0.001$), age (HR 1.04; 95% CI 1.02–1.06, $P < 0.001$) and VTE risk (Khorana model-high, HR 3.3; 95% CI 1.18–9.16, $P = 0.022$) significantly correlated with the risk of mortality. The probability of survival was higher in pts with MPV $>$ 10th percentile by Kaplan-Meier method (log rank test=3.08, $P = 0.0021$).**Conclusion:** The pre-chemotherapy MPV value may be useful as a prognostic factor of VTE and the risk of mortality in patients with DLBCL.**Disclosure of Interest:** None declared.

PO126-TUE

A new generic risk score for predicting venous thromboembolic events in cancer patients receiving chemotherapyMuñoz A¹, Ziyatdinov A², Souto JC³, Soria JM² and Soria JM²¹*Oncologia Medica, Hospital Gregorio Marañón, Madrid;* ²*Unit of Complex Disease, IIB-Sant Pau;* ³*Haemostasi and Trombosis Unit, IIB-Sant Pau, Barcelona, Spain***Background:** Venous thromboembolism (VTE) is one of the major causes of cancer-associated mortality. Risk for developing VTE increases when in chemotherapy, mainly in the outpatient setting. Current risk scores for predicting chemotherapy-associated VTE have low/moderate discrimination capacity. These models use clinical parameters. Thrombo inCode (TiC) is a new tool for VTE risk prediction using an algorithm that combines a genetic risk score (GRS) with subject's VTE clinical risk parameters (cancer type and cancer disease status 'CDS', included).**Aims:** To evaluate whether TiC predicts better the risk for chemotherapy-associated VTE than Khorana score.**Methods:** A prospective, observational study including 111 patients with locally advanced or metastatic cancer (colon, stomach, pancreas and lung) receiving systemic outpatient chemotherapy. Patients are followed-up for 6 months. Three predictive models were compared: a) Khorana score; b) Khorana score plus CDS and c) TiC. Genetic variants included in TiC are FVL, PT, F5 rs118203906 and rs118203905, F12 rs1801020, F13 rs5985, SERPINC1 rs121909548, SERPINE10 rs2232698 and A1 blood group rs8176719, rs7853989, rs8176743, rs8176750. Clinical risk factors in TiC are age, sex, family history of VTE, BMI, smoking, diabetes, type of cancer and CDS. Prediction capacity of each model was assessed in terms of the discrimination (area under the receiver operating characteristic curve, AUC)**Results:** The incidence of VTE at 6 months was 26% (Mainly in patients with pancreas cancer-43% >, followed by colorectal). Khorana score had an AUC of 0.509 (95% CI 0.386–0.633), Khorana score + CDS prediction model had an AUC of 0.64 (95% CI 0.513–0.767). Discrimination increased significantly with TiC (AUC: 0.80; 95% CI 0.699–0.897), $P < 0.05$.**Conclusion:** We conclude that TiC predicts chemotherapy-associated VTE risk significantly better than Khorana score with CDS.

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Disclosure of Interest: None declared.

PO127-TUE

Thromboprophylaxis of ambulant pancreatic cancer patients: single centre experience utilizing the lessons from FRAGEM and CONKO-04

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Background: There is evidence that primary thromboprophylaxis (TP) with low molecular weight heparin (LMWH) in ambulatory advanced pancreatic cancer (aAPC) patients results in significant reduction of venous thromboembolism (VTE).

Aims: Single centre experience with LMWH in APC patients referred for chemotherapy.

Methods: Between May 2009 and Dec 2014, 67 patients with APC were referred to Castle Hill Hospital for treatment. All patients received prophylactic LMWH with weight (wt)-adjusted dalteparin (D). A simplified dosing schema (SDS) avoiding ultra-high dose ($200 \text{ u kg}^{-1} \text{ D}$) from 3-months schedule of FRAGEM and low dose ($40 \text{ mg enoxaparin maintenance}$) of CONKO-04 was used, i.e. 7500 u for $\text{wt} < 50 \text{ kg}$, $10,000 \text{ u}$ for $50\text{--}80 \text{ kg}$ and $12,500 \text{ u}$ for $\text{wt} > 80 \text{ kg}$. LMWH was stopped at clinical best interest, patient preference, serious adverse event (e.g. major bleed) or patient death. Data for cumulative all-site, symptomatic and incidental VTE (as-VTE) and bleeding while on D is submitted here. Survival data, predicted VTE score based on Khorana index and duration of LMWH has been collected and will be presented at the annual meeting.

Results: At the time of diagnosis, mean age of patients was 67 years, 58% were male, 95% had pathologic diagnosis (adenocarcinoma) and 73% had stage IV. Eleven patients (16%) had VTE at presentation and received D as secondary TP. Fifty six (84%) patients received SDS for primary TP. Most received gemcitabine based chemotherapy but 5 patients were offered best supportive care (only) along with SDS. Only 5 cumulative recurrent or breakthrough as-VTE events occurred in all patients (7%). Eight patients (12%) had clinically significant bleeding of which 6 were 'major' by ISTH criteria.

Conclusion: In this 'real world' experience of thromboprophylaxis in aAPC patients, we found a marked reduction of expected VTE incidence using SDS. We report a higher incidence of major bleeding than in FRAGEM which can be explained however by the distinctly longer than 3 months administration of SDS.

Disclosure of Interest: None declared.

PO128-TUE

Treatment of cancer associated thrombosis after the initial 6 month period

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Background: The treatment with low-molecular-weight heparin (LMWH) is recommended for patients with cancer associated thrombosis (CAT) during the first 6 months; the initial dose 200 IU kg^{-1} daily can be reduced to 2/3–3/4 after 1 month. The therapy should be reevaluated after 6 month and subsequent therapy using LMWH or warfarin is recommended indefinitely, unless the cancer is resolved or any major contraindications arise.

Aims: To analyze the usage of various treatment modalities in CAT patients after the initial 6 month period according to the course of cancer.

Methods: Eighty-seven patients with CAT were treated with LMWH during the initial period. The proportion of patients in whom LMWH was replaced by warfarin during the first 6 months and during the entire follow up, was analyzed in relation to the course of the cancer.

Results: Six months after the diagnosis of thrombosis 9/29 (31.0%) patients with complete remission (CR) of the cancer were treated with warfarin, while only 3/12 (25%), 0/9 (0%), and 1/13 (7.7%) of the patients with partial remission (PR), stable disease (SD) and progression, respectively, were treated with warfarin. Patients with CR and PR were more frequently treated with warfarin than patients with SD or progression ($P = 0.02$). Thirteen patients died during the first 6 months, and 8 patients were followed < 6 months. During the entire follow up, 25/30 (83.3%) patients with CR switched to warfarin, while only 5/12 (41.7%), 1/9 (11.1%), and 1/13 (7.7%) patients with PR, SD, and progression, respectively, were treated with warfarin. The proportion of patients with CR which switched to warfarin was higher, than the proportion of patients with PR ($P = 0.007$), with SD ($P = 0.0003$), and with progression ($P < 0.0001$).

Conclusion: The course of malignant disease is important for the choice of appropriate therapy of CAT after the initial period of LMWH therapy.

Disclosure of Interest: None declared.

Coagulation factor V, X, II, XIII – II

PO129-TUE

Defining the differential effects of novel anticoagulants: fXa versus fIIa inhibition on coagulation and inflammation

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Background: Thrombin is the key protease in regard to thrombus formation; the same is not true in regard to protease dependent signaling.

Aims: Hence, we postulate that inhibition of either fXa or fIIa may have comparable effects in regard to coagulation, but convey different effects in regard to inflammation and receptor dependent regulation of cellular effects.

Methods: WT mice were either treated with low and high dose of dabigatran or Rivaroxaban for 1 week and then were analyzed for tail bleeding assay or for arterial injury induced thrombosis formation and LAD ligation (ischemic/reperfusion) induced myocardial infarction.

Results: Rivaroxaban and dabigatran have comparable dose-dependent effects in regard to bleeding and thrombosis in *in vivo* models. However, while fIIa inhibition abrogates the anti-inflammatory effect of fIIa already at low dosages, e.g. at dosages at which bleeding time is already prolonged, this is not the case when using the fXa inhibitor Rivaroxaban. Although Infarcted heart areas were similar in both groups, fxa inhibition abolished proinflammatory cytokines, IL-6, TNF-alpha and macrophages infiltration in infarcted heart tissue. In addition we observed higher levels of blood plasma endogenous activated protein C (aPC) in fXa inhibition group, as compared to in fIIa inhibition group. Western Blot analysis revealed NF- κ B levels significantly less in fxa inhibited group as compared to fIIa inhibition.

Conclusion: Taken together, these results strongly support that inhibition of fIIa and fXa have similar profiles in regard to the regulation of hemostasis, but differ in their ability to modulate the inflammatory response, with fXa inhibition being superior.

Disclosure of Interest: None declared.

PO130-TUE

Rivaroxaban associates with factor Xa faster than apixaban: a potential explanation for their different effects on tests of coagulationYeh C^{1,2}, Dale B³, Leslie B², Kim PY², Fredenburgh JC¹, Hirsh J¹, Eikelboom J¹ and Weitz JI¹¹Medicine; ²Thrombosis and Atherosclerosis Research Institute, McMaster University, Hamilton, Canada; ³Sansom Institute for Health Research, University of South Australia, Adelaide, Australia

Background: Rivaroxaban and apixaban are small molecules that reversibly bind the active site of factor (F) Xa with comparable affinity and inhibit its activity. Surprisingly, apixaban has less of an effect on the prothrombin time (PT) and activated partial thromboplastin time (aPTT) than rivaroxaban.

Aims: Although rivaroxaban and apixaban do not require routine monitoring, there are situations where measuring their anticoagulant effects is helpful. Therefore, we set out to explain why the two inhibitors have different effects on the PT and aPTT.

Methods: We measured the affinities of rivaroxaban and apixaban for FXa in purified and plasma systems, and compared their effects on (a) the PT, aPTT, and thrombin generation, (b) rate of association with FXa, and (c) prothrombin activation by prothrombinase in a purified system.

Results: Rivaroxaban and apixaban inhibited FXa with K_i values of 0.5 ± 0.1 and 0.6 ± 0.04 nM, respectively ($P = 0.3$), indicating that they bind FXa with similar affinities. However, rivaroxaban inhibited FXa at a 3-fold faster rate than apixaban (4.5 ± 0.4 and 1.5 ± 0.3 $\mu\text{M s}^{-1}$, respectively; $P < 0.001$); a difference that persisted when components of the prothrombinase complex were added and was also observed in recalcified plasma. In a buffer system, rivaroxaban was 2.6-fold more potent than apixaban at blocking prothrombin activation by prothrombinase (K_i values of 2.5 ± 0.2 and 6.5 ± 1.3 nM, respectively; $P = 0.02$). Likewise, in thrombin generation assays in plasma, rivaroxaban prolonged the lag time and suppressed peak thrombin to a greater extent than apixaban.

Conclusion: These findings reveal that despite their similar affinities for FXa, rivaroxaban associates with the enzyme more rapidly than apixaban and attenuates thrombin generation to a greater extent. These differences may explain why rivaroxaban prolongs the PT and aPTT more than apixaban.

Disclosure of Interest: None declared.

PO131-TUE

Generation of a recombinant truncated activated factor X as anti-hemophilic agentAbache T¹, Dezetter A-S¹, Longue A¹, Grenier D¹, Bourdon C¹, Dagher M-C², Marlu R², Fontayne A¹, Polack B² and Plantier J-L¹¹Dir Innov Therapeutique, LFB Biotechnologies, Loos; ²TIMC-IMAG ThEREX, CNRS, La Tronche, France

Background: A plasma derived activated factor X devoid of its gamma carboxylated-domain (gla) was demonstrated to restore coagulation in hemophilic plasmas. The molecule is likely acting in alleviating the TFPI inhibition of the coagulation initiation.

Aims: To study its mechanism of action and assess its properties *in vivo*, a recombinant form of the molecule was produced.

Methods: The cDNA of the molecule was optimized in codon for a human expression, fused to the synthetic signal peptide MB7 and cloned in a high efficiency vector (OptiHEK). A furin cleavage site was inserted between the light- and the heavy chain. A HEK 293F stable cell line expressing around $8\text{--}10$ $\mu\text{g mL}^{-1}$ of protein of interest was established. The molecule was co-expressed with the human furin

enzyme and produced in batch mode for up to 9 days. The furin level needs to be finely controlled since its overexpression led to a truncation of the heavy chain with the loss of around 5 kDa of material.

Results: Cell conditioned media demonstrated the ability to normalize hemophilic plasmas (hemophilia A/B and hemophilia A/B with inhibitors) and to possess FXa chromogenic activity. The protein was then purified using a heparin column followed by a benzamidine column. Due to the presence of contaminating cleaved product, the resulting product loses a percentage of its chromogenic activity but kept its ability to correct FVIII deficient plasmas. Thrombin generation assays were performed following an induction with tissue-factor (0.5 pM) or cephalin. Following each induction, the correction intervened with a shortened lag phase and followed a dose response. The truncated FX molecule (2 $\mu\text{g mL}^{-1}$) was able to generate an amount of thrombin (peak = 108 nM; ETP = 1571 nM) equivalent to a FVIII correction between 0.1 U mL^{-1} (peak = 79 nM; ETP = 1030 nM) to 1 U mL^{-1} (peak = 225 nM; ETP = 1538 nM).

Conclusion: The purified material is now ready to be assayed in the other hemophilic plasmas before being assayed in FVIII deficient mice.

Disclosure of Interest: T. Abache Employee of: LFB Biotechnologies, A.-S. Dezetter Employee of: LFB Biotechnologies, A. Longue Employee of: LFB Biotechnologies, D. Grenier Employee of: LFB Biotechnologies, C. Bourdon Employee of: LFB Biotechnologies, M.-C. Dagher Grant/Research Support from: LFB Biotechnologies, R. Marlu Grant/Research Support from: LFB Biotechnologies, A. Fontayne Employee of: LFB Biotechnologies, B. Polack Grant/Research Support from: LFB Biotechnologies, J.-L. Plantier Employee of: LFB Biotechnologies.

PO132-TUE

Phenotypic and genotypic characterization of severe factor VII deficiency patients in Central China

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Background: Hereditary factor VII deficiency (FVIIID) is a rare autosomal dominant inherited bleeding disorder related to a variety of mutations or polymorphisms throughout the factor VII gene (*F7*).

Aims: The aim of this study was to characterize the molecular defect of *F7* gene in 7 unrelated patients with severe FVIIID and to find genotype-phenotype correlation.

Methods: Mutation detection was performed by sequencing the whole *F7* gene coding region, exon-intron boundaries and 5' and 3' untranslated regions. Family studies were also performed. Multiple sequence alignment among species and vitamin K-dependent serine proteases was performed using DNAMAN. Online softwares were used to confirm the pathogenic of the missense mutation.

Results: We identified 11 unique lesions, including 7 missense mutations, 4 splice junction mutations, among which three were reported for the first time (p.Glu280Ala, p.Phe388Cys, c.64 + 1G>A). Multiple sequence alignment using DNAMAN analysis revealed that novel missense mutations were located in residues that highly conserved among species and vitamin K-dependent serine proteases. Homozygous state was seen in two unrelated patients, with or without Consanguinity. Others were compound heterozygotes of two mutations. Null mutations were identified in both alleles of patient 7 who died of CNS in the neonatal period, and who is the only patient who died from FVII deficiency in this report.

Conclusion: The majority of missense mutations reported in this study were located in the trypsin-like serine protease region (five out of six, p.Thr241Asn, p.Glu280Ala, p.Phe388Cys, p.Ser423Ile and p.Gly425Cys), suggesting that real attention should be paid to identify genetic defects in this region, which may also apply to other vitamin K

dependent factors. FVII deficiency patient with null mutation in both alleles shows severe bleeding symptoms.

Disclosure of Interest: None declared.

PO133-TUE

Fragment-2 inhibition of prothrombinase reveals a unique enzyme-substrate interface for the platelet-bound enzyme

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Background: Prothrombin is composed of fragment-1 which contains the Gla-domain, fragment-2, and a protease domain. During the hemostatic response, thrombin can cleave prothrombin at Arg155 to generate the Gla-domainless prethrombin-1 intermediate. Initial cleavage of Prethrombin-1 by membrane-bound prothrombinase generates fragment-2 and prethrombin-2, one or both of which inhibits PCPS- (75% phosphatidylcholine and 25% phosphatidylserine) but not platelet-bound prothrombinase.

Aims: To determine if the differential inhibition of prethrombin-1 activation by PCPS- vs. platelet-bound prothrombinase is due to the difference in substrate binding mediated by fragment-2.

Methods: Product inhibition during prethrombin-1 (1.4 μ M) activation was assessed by comparing progress curves of thrombin generation catalyzed by either PCPS- or platelet-bound prothrombinase (0.5 nM) with theoretical progress curves based on kinetic parameters ($K_m = 3.7$ vs. 3.2μ M and $V_{max} = 4.5$ vs. 5.5 nM s^{-1} ; PCPS vs. platelets, respectively). Fragment-2 inhibition was assessed by titrating fragment-2 into prethrombin-1 activation mixtures. Platelets were obtained from consenting human adults according to the Declaration of Helsinki.

Results: PCPS-bound prothrombinase catalyzed progress curves for prethrombin-1 (1.4 μ M, 25 °C, 1 h) but not platelet-bound prothrombinase did not recapitulate those defined by kinetic parameters, since 0.3 μ M vs. 0.06 μ M substrate remained in the PCPS- vs. platelet activation mixtures, respectively. Titration of fragment-2 also inhibited prethrombin-1 activation catalyzed by PCPS- but not platelet-bound prothrombinase.

Conclusion: Fragment-2 inhibits a critical binding interaction essential for the effective activation of prethrombin-1 by prothrombinase on PCPS- but not platelet-bound prothrombinase. This mechanism may ensure the efficient activation of prethrombin-1 generated at sites of injury while preventing its activation by prothrombinase bound to non-platelet surfaces.

Disclosure of Interest: None declared.

PO134-TUE

Novel F10: p.Cys57Arg mutation in a Pakistani severe FX-deficient proband

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Background: Hereditary deficiency of factor X (FX) is a rare coagulation defect. FX-deficient patients are still scarcely explored in Pakistan although rare bleeding disorders became quite common as a result of traditional consanguineous marriages.

Aims: The aim of the study was to give a first insight of FX gene mutations in Pakistani probands.

Preferences.

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Methods: Two severe FX-deficient patients from Pakistan were analyzed. Direct sequencing was performed on the coding regions, intron/exon boundaries and 5' and 3' untranslated regions of the *F10* gene.

Results: Both patients were 3 year-old with severe FX-deficiency (FX: C levels below 2%). They were from consanguineous marriages and presented with severe clinical symptoms including umbilical cord, hemarthrosis, bleeding after trauma and bruises. In the first case, *F10* genotyping revealed the p.Ala15Asp mutation at the homozygous state. This mutation has already been reported from India. The second case was a compound heterozygote for the p.Gly420Arg and the p.Cys57Arg mutations. The p. Gly420Arg mutation was previously reported from Costa Rica. The p.Cys57Arg is newly reported. Moreover, the Cys57 residue makes a disulfide bond with Cys62 suggesting a probably damaging effect of the p.Cys57Arg mutation.

Conclusion: Here, we report the first molecular analysis in Pakistani proband with a novel mutation in a small series of FX-deficient patients.

Disclosure of Interest: None declared.

Coagulation factor VIII and Factor IX – II

PO135-TUE

The IL-10 polarized cytokine pattern in innate and adaptive immunity cells contribute to the development of FVIII inhibitors

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Background: Hemophilia A (HA) is a bleeding disorder characterized by a qualitative or quantitative deficiency of factor VIII (FVIII). Antibodies against FVIII (inhibitors) block the procoagulant activity of FVIII are present in 10–15% of HA patients. The mechanism underlying the immunological events behind the development of inhibitors remains unknown.

Aims: This study aimed to understand immune response to FVIII in patients with HA who were positive [HA α -FVIII(+)] ($n = 25$) or negative [HA α -FVIII(-)] ($n = 30$) for inhibitors. Healthy blood donors were included in the study as controls ($n = 30$).

Methods: The study was conducted in Fundação Hemominas, Belo Horizonte, Minas Gerais, Brazil. The recruitment of participants and the methodology of the study were approved by the Brazilian ethics committees. Cytokine profiles [interferon- γ (IFN- γ), tumor necrosis factor- α (TNF- α), interleukin-4 (IL-4), IL-5, and IL-10] of participants' peripheral blood leukocytes were characterized by intracellular staining and flow cytometry.

Results: Presence of inhibitors was significantly associated with decreased frequencies of TNF- α ⁺ monocytes and neutrophils, IL-5⁺ monocytes, IL-4⁺ neutrophils, and increased frequencies of IL-10⁺ neutrophils and T cells. T cells from HA α -FVIII(-) expressed increased levels of almost all cytokines. In contrast, HA α -FVIII(+) showed lower levels of all cytokines in CD4⁺ and CD8⁺ T cells, except IL-10. B cells from HA α -FVIII(-) expressed increased levels of IL-4 while those from HA α -FVIII(+) expressed increased levels of IL-10. The global cytokine profiles of innate and adaptive immune cells showed an anti-inflammatory/regulatory pattern in HA α -FVIII(+)

and a mixed pattern, with a bias toward inflammatory cytokine profile, in HA α -FVIII(-).

Conclusion: The occurrence of these profiles seems to be associated with presence FVIII inhibitors and could be used in the future as biomarkers for FVIII inhibitors development. This study received financial support of Fundação Hemominas, Fapemig and CNPq.

Disclosure of Interest: None declared.

PO136-TUE

Evaluation of N8-GP long term safety in immune deficient rats

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Background: N8-GP is a 40 kDa PEGylated human recombinant coagulation FVIII, intended for the treatment of Haemophilia A. Conducting chronic toxicity studies in animals using human coagulation factors is challenged by anti-drug antibody development. Attachment of a PEG moiety to a therapeutic drug has been reported in some cases to induce vacuole formation in various tissues in animals after long term dosing.

Aims: In order to evaluate the long term safety of N8-GP, toxicity studies of 26 and 52 weeks duration were conducted in immune deficient rats (Rowett Nude (CrI:NIH-Foxn1^{tmu}) – Athymic rat).

Methods: Rats were dosed i.v. every 4th day with 0; 50; 150; 500 or 1200 IU kg⁻¹ N8-GP. The following experimental observations were performed: clinical observations, body weight, food consumption, ophthalmoscopy, haematology, clinical chemistry, coagulation, urinalysis, toxicokinetics, antibody analysis, organ weights, macroscopic and microscopic examination of organs. Furthermore, an immuno-histochemical staining method (IHC) was used to examine the distribution of PEG in selected tissues.

Results: N8-GP was well tolerated and there was no difference between the 26 and 52 weeks dosing. None of the toxicity endpoints were affected and no treatment related vacuoles were observed. PEG was not detected by IHC. The studies in immune deficient rats were conducted in order to assess the long term effects of 40 KDa PEGylation of human recombinant coagulation FVIII. No adverse findings were observed at doses spanning 50–1200 IU kg⁻¹/fourth day.

Conclusion: The No Observed Adverse Effect Level for N8-GP in the studies was found to be 1200 IU kg⁻¹ every fourth day (highest dose tested) based on the absence of treatment related adverse findings evaluated by standard toxicological endpoints and complemented by IHC staining for PEG. The immune deficient rat proved suitable for chronic toxicity studies and could be considered in cases where a model is needed for chronic toxicity testing of human proteins found to be immunogenic in animals.

Disclosure of Interest: None declared.

PO137-TUE

Association of arterial and venous thrombosis with clotting factor used in patients with hemophilia

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Background: A deficiency of coagulation factor VIII or IX exerts a protective effect on the development of thromboembolism. However, it remains unclear whether arterial and venous thromboses in persons with hemophilia (PWH) are associated with coagulation factor use.

Aims: This study evaluated the association between arterial and venous thrombosis and coagulation factor use among 1099 male hemophilia patients.

Methods: We used data collected from Taiwan's National Health Insurance Research Database between 1997 and 2010.

Results: There were 9 PWH with ischemic stroke, and 27 with acute coronary artery syndrome, including 2 myocardial infarction and 25 angina pectoris. Only 2 patients developed venous thrombosis during the study period. The dosage and frequency of clotting factor concentrate use among PWH who experienced thrombotic events were not significantly increased within 30 days prior to the occurrence of the event in comparison to their previous use, $P = 0.61$ and $P = 0.77$, respectively.

Conclusion: The study indicated clotting factor use is not associated with arterial or venous thrombosis among PWH.

Disclosure of Interest: None declared.

PO138-TUE

Assessing the indication and success of treatment for haemophilia patients: results from a delphi panel

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Background: Primary prophylaxis (PPX) with regular factor injections from early childhood is a generally acknowledged treatment goal in haemophilia A and B. PPX aims to prevent bleeding episodes and can lead to long-term complications (i.e. joint damage and disability). International guidelines encourage practice harmonization, but management of haemophilia is complex and differs across treatment settings and geographic regions.

Aims: To investigate which level of consensus could be established among haemophilia treaters around which baseline patient characteristics influence treatment decisions and what evaluation criteria determine if a treatment regimen is successful.

Methods: Three consecutive rounds of a Delphi survey were circulated to 15 haemophilia treaters in 12 countries. Initially, participants listed and prioritized characteristics they consider when deciding a treatment regimen for a new-to-them patient; evaluation criteria for treatment success and threshold values for the evaluation criteria for on-demand (OnD) and PPX patients. Subsequently, participants ranked these characteristics. Consensus on the relative importance of each characteristic was defined as 70% agreement.

Results: Consensus was reached on 11 of 21 listed baseline characteristics for deciding a treatment regimen for a new patient. The most important criterion according to panelists was inhibitor status. All four evaluation criteria for determining the success of treating an acute bleed reached consensus, as did 4 of 7 for an OnD over time treatment regimen. Only 4 of 15 criteria for determining the success of a PPX regimen reached consensus, among which the most important criterion was frequency of spontaneous joint bleeds.

Conclusion: Our study demonstrates that while PPX treatment in patients with haemophilia A or B are considered a standard to achieve, there is a lack of consensus about which criteria are most important for assessing the achievement of successful prophylaxis.

Disclosure of Interest: A. Iorio Grant/Research Support from: Novo Nordisk A/S and Bayer AG, Consultant for: Pfizer Inc, A. Rentz Employee of: Evidera, Z. Balantac Employee of: Evidera, C. Hoxer Employee of: Novo Nordisk A/S.

PO139-TUE

Assessing the burden of disease in haemophilia patients: a qualitative study

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Background: Haemophilia, a rare hereditary haemorrhagic disease, requires administration of regular clotting factor replacements to prevent and treat bleeds. Management of haemophilia places a considerable burden on patients and their caregivers.

Aims: Assess the burden of haemophilia in U.S. adolescent patients and primary caregivers.

Methods: IRB approval was obtained for this qualitative study. Male, moderate to severe haemophilia A or B patients receiving prophylaxis, aged 13 to 23 ($n = 15$), and their primary caregivers ($n = 7$) were recruited in Chicago, Illinois. Interviews were recorded Oct.–Nov. 2014, collated, and themes extracted using thematic text analysis.

Results: The burden degree expressed with having haemophilia varied and appeared to change with age. Older patients appeared more tolerant of bleed-associated issues. Patients were mixed about being open with others about having haemophilia. Adolescents struggling with openness preferred to be socially isolated. Embarrassment, perceptions of being handicapped and fear of stigmatization were common feelings among struggling patients. Caregiver perceptions of bleed frequency and pain levels were lower than patient reported, suggesting patients were not fully transparent. Caregivers reported higher stress levels caring for a child with haemophilia than caring for their unaffected siblings. Caregivers altered lifestyles to accommodate their perceived needs of the afflicted child; functional limitations (e.g., participation in contact sports) and overall quality of life impact were particular frustrations. Some caregivers did not expand their families for fear of having another child with haemophilia.

Conclusion: Haemophilia impacts patients with moderate to severe haemophilia and their caregivers at varying degrees. Impact nature differs among age groups. Patients embrace and cope more with their disease as they mature, however it is still evident that their condition influences their lifestyle choices.

Disclosure of Interest: U. Slothuus Employee of: Novo Nordisk A/S, M. Field Consultant for: OptumInsight Life Sciences, Inc., C. Hoxer Employee of: Novo Nordisk A/S.

PO140-TUE

Comparison of recombinant factor VIII products with respect to HMWP content and general heterogeneity

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Background: Blood clotting factor VIII (FVIII) is a complex 2332 amino acid protein with the domain structure A1-A2-B-A3-C1-C2. The mature FVIII molecule circulates as a heterodimer consisting of a heavy and a light chain held together by a metal ion. The B-domain is not needed for haemostatic function and different forms of recombinant FVIII have been produced including full length and B-domain truncated/deleted versions.

Aims: Turoctocog alfa (B-domain truncated form) and three commercial recombinant FVIII products (two full length and one B-domain deleted form) have been compared with respect to the presence of HMWP species and general heterogeneity of the rFVIII protein.

Methods: The HMWP content of the rFVIII products was elucidated by Static Light Scattering (SLS)-SE-HPLC and the general heterogeneity of the products was examined by RP-HPLC fractionation followed by mass spectrometry, peptide mapping and N-terminal sequencing.

Results: Static Light Scattering (SLS)-SE-HPLC results demonstrate that for a B-domain deleted product the estimated molar masses of the eluting species are higher than expected due to interference from High Molecular Weight Protein (HMWP) species. Average molar masses of 300 and 323 kDa are obtained for the main peak of the full length products, respectively. The SLS data support that LMWP peaks observed correspond to rFVIII species with heterogeneously processed B-domains. With respect to the general heterogeneity full length recombinant FVIII products display a significantly higher heterogeneity with fragmentations in the B-domain region and free light chain accounting for most of this.

Conclusion: It is demonstrated, that full length recombinant FVIII proteins in general display a significantly higher heterogeneity when compared to B-domain deleted/truncated proteins. The heterogeneity includes HMWP species, the presence of free light chain and fragments formed following cleavages in the B-domain.

Disclosure of Interest: None declared.

PO141-TUE

Development of an ELISA for the detection of binding antibodies against human coagulation factor VIII in rat plasma

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Background: A new rat model of hemophilia A (HA) has recently been developed, but the antibody response to coagulation factor VIII (FVIII) products in this model have yet to be investigated. A specific rat anti-FVIII antibody assay is required to understand the full antibody spectrum during an immune response towards administered FVIII.

Aims: Develop an enzyme-linked immunosorbent assay (ELISA) for detection of binding antibodies against human FVIII in rat plasma, for fast screening of multiple samples and with the potential of immunoglobulin subclass identification.

Methods: Citrated plasma was obtained from rats after intravenous administration of recombinant B-domain deleted human FVIII (rhFVIII) ($n = 18$) and from untreated control rats ($n = 10$). Plasma anti-hFVIII antibodies were captured on a rhFVIII-coated 96-well plate and detected by HRP-conjugated polyclonal rabbit anti-rat-Ig antibodies converting TMB chromogen. Assay qualification was performed using FDA and EMEA guidelines for specificity, precision, matrix effect, quality controls and assay cut-point calculations, and by comparison to an existing radioimmunoassay (RIA) for binding anti-rhFVIII IgG in rat plasma.

Results: Results were expressed as optical density (OD) at 1:160 sample dilution. Lower limit of detection (LLD) was OD 0.5 and lower limit of quantification (LLQ) was OD 1.0 ($2 \times \text{LLD}$). Intra- and inter-plate imprecision (CV) was 6.6% and 7.5%, respectively. Analytical specificity was confirmed by significant reduction in OD response after pre-incubation with rhFVIII. In comparison with RIA detection a significant correlation ($r^2 = 0.73$) and low rate of misclassification (5.9%) were observed.

Conclusion: A new ELISA has been developed for the detection of rat anti-human FVIII antibodies in rat plasma, for quick and easy large-scale identification of antibodies in immunogenicity studies with hFVIII products, with additional potential for antibody subclass differentiation.

Disclosure of Interest: K. Lövgren Employee of: Novo Nordisk A/S, K. Weldingh Employee of: Novo Nordisk A/S, K. Larsson Employee of: Novo Nordisk A/S, M. Kjelgaard-Hansen Employee of: Novo Nordisk A/S, B. Wiinberg Employee of: Novo Nordisk A/S.

PO142-TUE

The type of reagents determines the accuracy of calibration with a product specific standard for the measurement of the B-domain deleted FVIII Moroctocog alfa-AF activity

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Background: The measurement of the B-domain deleted FVIII Moroctocog alfa-AF can be difficult due to discrepancies between values obtained with one-stage and with chromogenic FVIII assays. To solve these issues, the use of a specific Moroctocog alfa-AF laboratory standard (RAFLS) is recommended.

Aims: Because of discordant reports regarding the usefulness of calibration with RAFLS, we investigated how the calibration with RAFLS and the measurement of post-infusion levels of Moroctocog alfa-AF are influenced by the reagents of the one stage FVIII assay.

Methods: Calibration curves were generated with RAFLS and normal plasma using different commercially available FVIII-deficient plasmas. The different calibrations were then used to determine FVIII levels in plasmas of patients treated with Moroctocog alfa-AF.

Results: With some FVIII-deficient plasmas, the coagulation times of given FVIII concentrations were longer with RAFLS than with plasma. Accordingly, Moroctocog alfa-AF post-infusion levels were up to 30% higher with the RAFLS calibration curves than with the plasma ones. The values determined using the RAFLS calibration curves were close to the values determined with a chromogenic assay. Unexpectedly, with other FVIII-deficient plasmas, the coagulation times obtained with RAFLS were shorter than with plasma. The use of RAFLS calibration curves with these one-stage assay reagents led to a two-fold underestimation of Moroctocog alfa-AF activity by comparison to the levels determined with the one-stage calibrated with plasma or with a chromogenic assay.

Conclusion: Depending on the reagents of the one-stage FVIII assay, calibration with RAFLS can reduce or increase the difference between Moroctocog alfa-AF activities measured in plasma samples with the one-stage and with the chromogenic FVIII assays. Appropriate reagents should therefore be selected to measure Moroctocog alfa-AF post-infusion levels with a one-stage FVIII assay calibrated with the corresponding laboratory standard.

Disclosure of Interest: None declared.

PO143-TUE

Health-related quality of life in children with haemophilia from Turkey

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Background: Haemophilia is a chronic bleeding disorder.

Aims: The aim of this study is assessment of quality of life (QoL) in children with hemophilia in Turkey and describing relationship between children's QoL and depression and anxiety of parent and children.

Methods: Thirty-four children and adolescents aged between 4 and 16 years with haemophilia without inhibitors were included in the study. Turkish version of the Hemophilia-Specific Quality of Life Index (Haemo-QoL) questionnaire was administered to the pediatric patients.

Results: In terms of Haemo-QoL, among children aged 4–12 years were impaired mainly in the dimension of family, sport/school, whereas among adolescent aged 13–16 years had higher impairments in the dimension of physical health. Among adolescents, QoL impairment in dimension of physical health, sport and school are significantly higher than children. Children aged 4–7 years reported worse QoL score in dimension's family and treatment when compared children and adolescents aged 8–16 years. Children whose mothers has middle or high rates of depression reported significantly more impairment in terms of family dimension and Total QoL. Among Children whose mothers has middle or high levels of anxiety was detected more impairment about dimension of physical health, family, friend, others. In our study, mothers depression scores were positively correlated with values of total QoL. Also, anxiety scores of the mothers were positively correlated with dimension's physical health, friend and others.

Conclusion: In our study, among children aged between 4 and 12 years, impairments of family dimension may be due to over protective attitude by the parents. Impairments of the sport/school dimension in children and the physical health dimension in adolescent can be related to arthropathy, hematoma and/or pain which may result from hemophilia. Anxiety and depressive symptoms of mothers may be associated chronic stress on account of the chronic disease.

Disclosure of Interest: None declared.

PO144-TUE

Structural characterisation of recombinant factor IX fusion protein linked with human albumin

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Background: CSL has developed a recombinant Factor IX therapeutic drug with a prolonged half-life consisting of a fusion protein of human coagulation Factor IX with human albumin (rIX-FP). The recombinant fusion protein has completed late stage clinical trials for treatment of haemophilia B and in Dec 2014 the BLA was submitted to the FDA.

Aims: Protein structure and post-translational modifications (PTMs) are critical for functional efficacy of coagulation factors. The molecular structure of CSL's recombinant Factor IX fusion protein was fully characterized here, and compared with commercially available plasma Factor IX (FIX) and recombinant FIX (BeneFIX®).

Methods: PTMs and O-linked glycans were analysed by peptide mapping and mass spectrometry, N-linked glycans were characterised by glycosidase digestion, chromatography, mass spectrometry.

Results: The fusion protein rIX-FP contains 28 disulfide bonds, 11 in the FIX domain, 17 in the albumin domain, which were confirmed to exist in their predicted linkages. Gamma carboxylation was confirmed, and O-linked glycans on the light chain were characterized. The PTMs on the activation peptide of rIX-FP were compared to those in plasma and recombinant FIX. The activation peptide of the rIX-FP is highly similar to that of recombinant FIX, and similar structures are found on plasma FIX. The N-linked glycoforms of rIX-FP are complex-type glycans and include sulfated forms. No Gal alpha1-3 Gal or high mannose forms were detected. High resolution mass analysis of the protease- and albumin domains from the fusion protein confirmed the absence of unexpected PTMs.

Conclusion: All disulfide bonds in the fusion protein rIX-FP are as expected from the parental proteins, FIX and albumin, consistent with a higher order structure of two separate domains. The PTMs in rIX-FP are comparable to recombinant and plasma FIX, thus supporting its suitability as a therapeutic agent.

Disclosure of Interest: A. Meister Shareholder of: CSL Limited, Employee of: CSL Limited, N. Grammel: None declared, M. Pelzing: None declared.

PO145-TUE

Tissue factor exerts factor Xa-catalyzed factor VIII activation in the presence of von Willebrand factor in a factor VIIA-independent mechanism

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Background: In the cell-based coagulation model, factor (F)VIIa complex with tissue factor (TF) initiates the blood coagulation by generating FXa as extrinsic Xase complex and activates FIX which composes intrinsic Xase complex (in-Xase). We reported that FVIIa/TF directly activated FVIII in an early timed-phase (Soeda, JTH, 2010), and TF enhanced the in-Xase activity through possible interaction with FVIIIa (Ogiwara, ASH, 2010).

Aims: We developed the study on the association with FVIII and TF in the presence of von Willebrand factor (VWF).

Methods: A direct binding of TF to FVIII was performed by SPR-based assays (BIAcore®) and solid phase-based ELISA. Effects of TF on FXa-catalyzed activation and proteolysis of FVIII ($\pm$ VWF) was examined with one stage-clotting assay and SDS-PAGE/western blot.

Results: An SPR-based assay revealed the direct binding of FVIII to immobilized non-lipidated TF (non-TF; Altor BioScience) with higher affinity (K_d ; 0.13 ± 0.01 nM). An ELISA also showed that non-TF bound to immobilized FVIII, although the binding affinity was modestly weaker (K_d^{app} ; 8.1 ± 1.2 nM). In addition, soluble TF (Altor BioScience) as well as lipidated TF (L-TF; Dade Innovin®) bound to FVIII (K_d^{app} ; 21.9 ± 3.4 , 3.6 ± 0.4 nM, respectively). The presence of VWF significantly inhibited the FVIII and L-TF binding by ~80%. We further assessed the functional potentials of TF on FVIII/VWF interaction during coagulation process. FXa-catalyzed activation of FVIII showed an ~four-fold peak level of initial, but that of FVIII/VWF was little observed. The addition of L-TF, however, increased FVIII activity by ~1.8-fold in the presence of VWF dose-dependently. SDS-PAGE revealed that the addition of L-TF markedly accelerated FXa proteolysis at Arg³⁷² in FVIII complex with VWF, despite little proteolysis in the absence of TF.

Conclusion: FVIII forming a complex with VWF could be activated by FXa under the presence of TF, and in-Xase complex appeared to exert in an early timed-phase of coagulation process before thrombin generation.

Disclosure of Interest: None declared.

PO146-TUE

Physicochemical characterization of recombinant single-chain factor VIII (rVIII-singlechain)

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Background: rVIII-SingleChain is a novel recombinant single-chain factor VIII (FVIII) construct, where the heavy and light chain are covalently linked. rVIII-SingleChain is expressed in Chinese Hamster Ovary (CHO) cells and purified by chromatographic methods. The tertiary structure of rVIII-SingleChain is determined by the polypeptide backbone including the respective disulphide linkages. In addition, its physicochemical properties are influenced by post-translational modifications (i.e. N-Glycosylation and Tyrosine sulphation). These parameters finally provide the basis for the pharmacological profile.

Aims: Because of the high relevance these physicochemical parameters were investigated in the present studies.

Methods: rVIII-SingleChain was investigated by liquid chromatography-electrospray ionisation-time of flight-mass spectrometry regarding primary structure, disulfide connectivity and Tyr-Sulfation. N-glyco-

sylation was determined by High pH anion exchange chromatography.

Results: The expected primary structure was confirmed as well as the predicted disulphide connectivity and the presence of three predicted unpaired cysteine residues. Additionally, a nearly complete sulphation of the six Tyrosine residues could be shown, in particular Tyr1680 (acc. to full-length FVIII numbering) relevant for the binding of rVIII-SingleChain to von Willebrand factor. The N-glycosylation pattern revealed mainly sialylated complex-type bi-, tri- and tetra-antennary structures and high mannose structures in accordance with other CHO cell-derived Factor VIII molecules.

Conclusion: In summary, it can be concluded that rVIII-SingleChain is a well-defined, homogenous FVIII product regarding its physicochemical attributes.

Disclosure of Interest: S. Schmidbauer Employee of: CSL Behring GmbH, L. Robbel Employee of: CSL Behring GmbH, P. Sebastian Employee of: CSL Behring GmbH, R. Witzel: None declared, N. Grammel Employee of: GlycoThera GmbH, H. Metzner Employee of: CSL Behring GmbH, S. Schulte Employee of: CSL Behring GmbH.

PO147-TUE

FVIII activity of long-acting turoctocog alfa pegol (N8-GP) can be accurately and precisely measured by different commercial chromogenic assays

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Background: GlycoPEGylated recombinant FVIII (turoctocog alfa pegol; N8-GP) was developed to prolong the half-life of turoctocog alfa and potentially reduce dosing frequency. FVIII activity (FVIII:C) is commonly monitored using one-stage aPTT-based clotting assays. However, PEG-conjugation can affect clotting times in an aPTT reagent-dependent manner. MASAC has recently recommended FVIII chromogenic assays, subject to FDA approval, for the measurement of FVIII:C of PEG-conjugated FVIII products.

Aims: To evaluate the performance of N8-GP using commercial FVIII chromogenic assay kits adapted to comply with European Pharmacopoeia guidelines for potency labelling, and to evaluate the suitability of the kits for clinical monitoring.

Methods: Six FVIII chromogenic assay kits were investigated for potency assessment. Samples and WHO 8th international standard (IS) were pre-diluted in VWF-containing FVIII-deficient plasma and further diluted in buffer containing 1% albumin. FX activation times were assessed to achieve 50% activation. Four kits were adapted for the ACL TOP 500 (Instrumentation Laboratory) to measure simulated post-administration samples. Samples were prepared by spiking haemophilia A plasma with turoctocog alfa or N8-GP and assessed using two calibrators (WHO 8th IS and a human plasma calibrator).

Results: There was no significant difference in N8-GP potency among the kits. Mean FVIII:C ($508\text{--}594$ IU mL⁻¹) was within 25% of the labelled N8-GP potency (527 IU mL⁻¹). In simulated post-administration plasma samples, all kits were highly accurate and precise, with no significant difference between turoctocog alfa- or N8-GP-containing samples ($P > 0.05$). There was no significant difference between calibrators, although values obtained using the WHO 8th IS were closer to nominal values.

Conclusion: The results for potency assessment and clinical monitoring of N8-GP are similar for all chromogenic assay kits and support their use as the recommended assay for measuring FVIII:C.

Disclosure of Interest: None declared.

PO148-TUE

Molecular genotyping of haemophilia A in Saudi Arabian population: report of novel mutations

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Background: Hemophilia A is an X-linked bleeding disorder caused by mutations in the Factor VIII gene. Molecular testing for the Factor VIII gene is difficult due to its large size. More than 900 different mutations have been described in Factor VIII gene.

Aims: To investigate the Factor VIII gene mutations in Saudi Arabian population.

Methods: For genotyping Factor VIII a cohort of 107 samples from 87 of families undergoing treatment were collected. All patients were tested for Factor VIII coagulant activity on Behring Coagulation System. Genomic DNA was isolated from blood on MagNapture system. Screening for inversion 1 (Inv-1) and inversion 22 (Inv-22) were done by the methods of Bagnall et al., 2002 and Rossetti et al., 2005 respectively. PCR products from the family's index cases were sequenced on ABI 3500 Genetic analyzer.

Results: Out of 107 cases, we found 2 samples were positive (affected) for Inv-1 and 17 cases were positive (15 affected and 2 carriers) for Inv-22. Out of 32 samples (32 families) we have sequenced for selected exons of Factor VIII, we have found two novel mutations (missense) c.99G>T, p.(W33C), and c.355G>C, p.(A119P) in exons 1 and 3 respectively; and one novel deletion (frame shift mutation) c.6482DelC, p.(P2161Lfs*25) in exon 23; and a novel mutation at splice junction (c.6430 –1G>A, Splicing acceptor site exon 23) in each patient respectively. We have also found 3 known mutations in 5 individual patients. Two of the patients had a previously reported mutation in exon 4, one patient had a known mutation in exon 6, and 2 patients had a known mutation in exon 12. Genotype-phenotype correlations and computer prediction analysis on the effect of missense mutations on the secondary structure of the Factor VIII protein are performed and the relationships evaluated.

Conclusion: We have established Haemophilia A genotyping in Saudi Arabian population. The novel mutations we found here, and its molecular dynamic simulation results to predict the deleterious effects will be discussed.

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PO149-TUE

Different strategies for treatment on hemophilia patients with inhibitor, costs versus benefits

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Background: Haemophilia A and B is an X-linked disorder with an incidence of 1/5000 and 1/20,000 males, respectively. It results from low or absent factor VIII (FVIII) and factor IX (FIX), respectively. The mainstay of treatment is factor replacement. Between 20% and 35% of patients with haemophilia A and between 4% and 6% of those with haemophilia B develop inhibitory antibodies.

Aims: The aims of this study were to reassess and review the most recent publications concerning the costs of inhibitor treatment by focusing on the outcome, success rate, the duration of studies and other parameters such as number of doses, method of investigation and time elapsed before initiation of treatment.

Methods: The keywords that we used for our search were inhibitor, haemophilia A, costs, cost analysis and ITI. We found a total of 63 studies of after a review of the abstracts, and five were excluded after full text review, leaving 12 (eight for bypassing agents and four for ITI) that were suitable for our research.

Results: ITI is the standard treatment to cure patients of inhibitors. A direct comparison of cost is not possible between studies because of variables such as body weight, number of bleeding episodes, treatment options, dosage regimen and prices. As indicated by the two long-term studies, ITI-treatment is expensive in the short-term treatment, but in the long term, it becomes affordable.

Conclusion: Bypassing agents are the mainstay of bleeding treatment. Cost is an important issue. Our review did not show any priority between the products. Cost can be influenced by many elements such as price, availability and different regimens. More important is the individual response rate, half-life, complications, type of bleeding/situation (mild to severe) or whether they are going to be used during surgery. Further studies are needed using fixed doses and a clear definition of treatment success.

Disclosure of Interest: None declared.

PO150-TUE

A plasma- and cell-based thrombin generation assay to assess the anticoagulant effect of tissue factor pathway inhibitor on endothelial cells

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Background: Thrombin generation (TG) is a fundamental part of the clotting cascade. *In vitro*, TG is commonly monitored in platelet-poor plasma by calibrated automated thrombography (CAT). However, CAT usually does not assess the influence of cellular components on TG. Tissue factor pathway inhibitor (TFPI) is the major physiological regulator of extrinsic coagulation. TFPI circulates in plasma and is present on the vascular cell surface.

Aims: The goal was to develop a plasma-based TG assay in 96-well format that incorporates human umbilical vein endothelial cells (HUVECs) as an exploratory tool to study cell-based TFPI, a target protein for the development of new hemophilia treatment options.

Methods: Adherent HUVECs at an optimized cell density were stimulated with tumor necrosis factor (TNF-α) to induce low levels of tissue factor (TF) expression. After adding normal or TFPI-depleted human plasma, TG was monitored without adding exogenous TF or phospholipids (PLs) in a fluorescence reader. In some experiments, TFPI inhibitory peptides or antibodies were used to demonstrate the TFPI dependency of the assay.

Results: The cell-based TG assay incorporating HUVECs as the only source of TF and membrane was shown to be sensitive and robust, with a variability of <20% CV for the main CAT parameters. At the established conditions, HUVEC-TFPI made a major contribution (~80%) to the total anticoagulant TFPI effect, although plasma TFPI is in high excess to HUVEC-TFPI. This is likely due to the local TF stimulation and restriction of coagulation to the HUVEC surface.

Conclusion: The HUVEC/CAT assay shows a substantial contribution of HUVEC-derived TFPI to the total anticoagulant effect of TFPI when cell-derived TF serves as coagulation trigger, which specifically allows assessment of the effect of TFPI-targeting substances on cellular TFPI. Further studies are planned to evaluate the contribution of platelets to TFPI-regulated TG.

Disclosure of Interest: G. Gerstenbauer Employee of: Baxter Innovations GmbH, S. Knappe Employee of: Baxter Innovations GmbH, E. Panholzer Employee of: Baxter Innovations GmbH, K. Prohaska Employee of: Baxter Innovations GmbH, F. Knofl Employee of: Baxter Innovations GmbH, N. Ullrich Employee of: Baxter Innovations GmbH, R. Pachlinger Employee of: Baxter Innovations GmbH, F. Scheiflinger Employee of: Baxter Innovations GmbH, M. Dockal Employee of: Baxter Innovations GmbH.

PO151-TUE

Lethality of mouse tissue factor pathway inhibitor gene disruption is rescued by transgenic human tissue factor pathway inhibitor knock in

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Background: Tissue factor pathway inhibitor (TFPI) is a key regulator of the extrinsic coagulation pathway. Deletion of the first Kunitz domain of TFPI in mice is known to be incompatible with viability due to intrauterine lethality.

Aims: To generate transgenic humanized TFPI mice in which mouse (m)-TFPI is entirely replaced by human (hu)-TFPI, to facilitate analysis of specific anti hu-TFPI antagonists without interference of m-TFPI.

Methods: Integration of the targeting vector and subsequent breeding analysis was followed by genomic PCR. Expression analysis of hu-/m-TFPI a and b mRNAs was performed by reverse transcription, cloning, and sequencing of the obtained DNA fragments. Protein levels of hu- and m-TFPI in plasma of transgenic and wild-type (wt) mice were analyzed by species specific ELISAs.

Results: Homozygous humanized TFPI mice were viable and exhibited no obvious abnormalities. They showed normal litter size with equal numbers of female and male pups. Genomic PCRs revealed proper integration of the targeting vector into the mouse chromosome and the homozygous status. Expression analyses of humanized TFPI mice on mRNA level demonstrated the absence of m-TFPI a. Alternative spliced m-TFPI b messages lacking the signal sequence were discovered, likely leading to a nonfunctional protein. Hu-TFPI a mRNA was detected in different tissues in the humanized TFPI mice. The TFPI protein level in plasma of humanized mice was below the detection limit of the ELISA and at least ~300 fold below the level of wt mice.

Conclusion: Low levels of hu-TFPI might compensate the function of m-TFPI *in vivo* and circumvent embryonic lethality. Furthermore, we established a new mouse model which allows the regulation of physiologic and pathologic pathways to be studied at TFPI below the limit of detection.

Disclosure of Interest: R. Pachlinger Employee of: Baxter Innovations GmbH, R. Hartmann Employee of: Baxter Innovations GmbH, A. Kolm Employee of: Baxter Innovations GmbH, E. Panholzer Employee of: Baxter Innovations GmbH, N. Ullrich Employee of: Baxter Innovations GmbH, F. Knofl Employee of: Baxter Innovations GmbH, M. Schuster Employee of: Baxter Innovations GmbH, W. Hoellriegl Employee of: Baxter Innovations GmbH, F. Scheiflinger Employee of: Baxter Innovations GmbH, M. Dockal Employee of: Baxter Innovations GmbH.

PO152-TUE

Longitudinal monitoring of TFPI levels and their correlation with coagulation parameters in healthy subjects: a one-year observation

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Background: Tissue factor pathway inhibitor (TFPI) is an important physiological regulator of coagulation by inhibiting the extrinsic coagulation pathway. Patients with Hemophilia A were found to have significantly lower full length (fl) TFPI levels compared with control patients (0.23 ± 0.06 nM vs. 0.36 ± 0.08 nM), suggesting that TFPI deficiency may also confer some protective effect in Hemophilia A (Duckers et al. 2008). Inhibition of TFPI improves hemostasis and may become a treatment option for patients with hemophilia including those with inhibitors. Longitudinal monitoring of the TFPI normal plasma level may help guide therapeutic/dosing decisions.

Aims: To investigate the intra- and inter-individual variation of TFPI over an extended period.

Methods: Citrated plasma samples from 38 healthy subjects (18 m/20f) aged 23–64 (average 35) years were collected at monthly intervals up to 14 times. Total and full length plasma TFPI was quantified by ELISA. Coagulation parameters were measured by calibrated automated thrombogram (CAT) at low TF triggering conditions.

Results: TFPI levels were significantly higher in males (1.17 ± 0.23 nM total, 0.42 ± 0.14 nM flTFPI) than in females (1.11 ± 0.22 nM total, 0.34 ± 0.12 nM flTFPI). Higher TFPI levels correlated with lower peak thrombin (122 ± 38 nM in males vs. 187 ± 71 nM in females) and endogenous thrombin potential (ETP) (1137 ± 222 in males vs. 1554 ± 349 in females). No seasonal variation in total and flTFPI levels was observed. On average, individuals' plasma flTFPI varied up to 32% from their respective mean TFPI concentration. In 92% of donors, the flTFPI level remained constant within 1.65-fold above or below the individual's mean over the observation period. No variation higher than three-fold was observed.

Conclusion: TFPI levels in plasma were shown to remain constant in individual donors over one year, indicating that drugs targeting TFPI may be useful in preventing bleeding in hemophilia patients without the need for a TFPI concentration tailored therapy.

Disclosure of Interest: R. Hartmann Employee of: Baxter Innovations GmbH, J. Koehn Employee of: Baxter Innovations GmbH, E. Panholzer Employee of: Baxter Innovations GmbH, B. Marchula Employee of: Baxter Innovations GmbH, E. Schoenegger Employee of: Baxter Innovations GmbH, B. Reipert Employee of: Baxter Innovations GmbH, F. Scheiflinger Employee of: Baxter Innovations GmbH, M. Dockal Employee of: Baxter Innovations GmbH.

PO153-TUE

Comparative study of full-length and B-domain modified RFVIII products shows no relation between B-domain modification and the content of subvisible particles

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Background: Immune responses to therapeutic products may pose problems for both patient safety and product efficacy. In recent years the authorities have shown increased focus on the potential immunogenicity of subvisible particles, which new techniques have made possible to analyse in a quantitative manner. It is therefore of interest to analyse rFVIII products with respect to the content of subvisible particles when administered to a patient.

Aims: To assess the amount and morphology of subvisible particles in four rFVIII products (two B-domain modified, one of them turoctocog alfa, and two full-length) in a clinically relevant setting.

Methods: Three batches of each product were prepared according to the prescribing information. A uniform and equal plunger pressure was applied for butterfly needle ejection. Micro flow imaging (MFI) analysis in three repetitions of each sample was performed to measure the number of subvisible particles (2–100 μm).

Results: MFI results showed very different counts of subvisible particles for the various rFVIII products. Turoctocog alfa had particle counts in the range 6000–8000 particles mL^{-1} , while the other B-domain modified product had particle counts in the range 30,000–190,000 particles mL^{-1} . The two full-length products had particle counts in the range 12,000–15,000 and 7000–40,000 particles mL^{-1} , respectively. Image analysis showed that some of the particles were silicone-like with a very distinct morphology. A software filter was subsequently applied to remove silicone-like particles from the data, which did not change the ranking of the rFVIII products with respect to the number of subvisible particles.

Conclusion: The study showed no correlation between the amount or morphology of subvisible particles and B-domain modification of rFVIII products in solutions ready for injection. Differences in production and product handling are more likely causes. Turoctocog alfa showed the lowest number of subvisible particles.

Disclosure of Interest: None declared.

PO154-TUE

Thrombin generation in human hemophilia plasma at reduced antithrombin levels and concomitant factor or bypass agent addition

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Background: ALN-AT3, a subcutaneously administered investigational RNAi therapeutic targeting antithrombin (AT), is currently being evaluated in persons with hemophilia in a Phase I study (NCT02035605). Based on its mechanism of action, ALN-AT3 could potentially offer benefit to persons with hemophilia A or B, with or without inhibitors.

Aims: As factor replacement is the standard therapy in hemophilia and as bypass agents (BPAs) are used to treat bleeds in hemophilia with inhibitors, we investigated the dynamics of thrombin generation in hemophilia plasma at varying doses of replacement factor and BPAs in the background of different levels of residual AT.

Methods: Human severe hemophilia A and hemophilia B donor plasma was obtained and immunodepleted of AT to varying levels. To these plasmas, replacement factor (rFVIII or rFIX) or BPAs (e.g. rFVIIa and aPCCs) were added at different concentrations to generate test plasmas representing a matrix of AT and factor/BPA levels. Thrombin generation measurements were made in the various test plasmas using the calibrated automated thrombogram (CAT) method.

Results: Both peak thrombin and endogenous thrombin potential (ETP, area under the thrombin generation curve) increased as AT decreased in hemophilia plasma, consistent with the therapeutic hypothesis that decreasing AT can increase thrombin generation in hemophilia plasma. However, thrombin generation levels did not exceed those associated with normal conditions (100% factor, 100% AT), even when AT was fully depleted in severe hemophilia plasma. Increasing factor or BPA dose increased thrombin generation in an additive manner on top of increases achieved with reduced AT.

Conclusion: These data demonstrate ranges of AT reduction and factor/BPA dose that result in enhanced thrombin generation without exceeding normal levels. In addition, the data suggest that in the back-

ground of reduced AT a substantially lower dose of replacement factor/BPA could potentially be used to achieve the same level of hemostatic effect.

Disclosure of Interest: A. Akinc Employee of: Alnylam Pharmaceuticals, A. Sehgal: None declared, K. Qian Employee of: Alnylam Pharmaceuticals, H. Prabhala Employee of: Alnylam Pharmaceuticals, O. Larsen: None declared, B. Sorensen Employee of: Alnylam Pharmaceuticals.

PO155-TUE

Rate-limiting roles of tenase complex of factors VIII and IX in platelet procoagulant activity and formation of platelet-fibrin thrombi under flow

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Background: While stable thrombus formation is known to rely on interactions between activated platelets and the coagulation system, the role of tenase activation in these processes is unclear.

Aims: We studied the role of tenase in the formation of stable platelet-fibrin thrombi under flow conditions.

Methods: With blood from FVIII or FIX deficient mice or from patients with severe haemophilia A or B, the role of platelet-stimulated tenase activity in the formation of platelet-fibrin thrombi on collagen was assessed. In addition, FVIII and FIX were localized on the thrombus with confocal microscopy.

Results: With tissue factor (TF) present, deficiency in FVIII or FIX suppressed thrombus growth, fibrin formation and platelet phosphatidylserine (PS) exposure. In either case, residual fibrin formation was eliminated in the absence of TF. These findings were confirmed with blood from a haemophilia A or B patient. The extrinsic way of coagulation contributes to platelet-controlled fibrin clot formation under flow at TF concentrations as low as 1 pM. Interestingly, considerable levels of TF (≥ 10 pM) are required to induce fibrin formation in blood from haemophilia patients, and even then the intrinsic way via tenase appears to enhance this process. In thrombi, PS-exposing platelets bound (activated) FIX and FX, but no more than limited amounts of FVIII, which preferentially accumulated on collagen fibers via von Willebrand factor. Activity of newly formed FXa and thrombin was confined to the sites of thrombi, and displayed the same kinetics. Direct FXa inhibitors, given to human blood, suppressed the thrombin and fibrin formation.

Conclusion: Our data point to a potent enforcement loop in thrombus formation of FX activation, subsequent thrombin and fibrin generation, leading to FXa-mediated stimulation of platelet PS exposure. Herein, the FVIII/FIX-dependent stimulation of platelet procoagulant activity can be a limiting factor for fibrin formation under flow conditions.

Disclosure of Interest: None declared.

PO156-TUE

Treatment of severe bleeding and prophylaxis with rFVIIa in a child with FVIII inhibitor

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Background: Hemophilic children with inhibitors are at risk for severe bleeding. Both the treatment and control of bleeding are expensive and sometimes difficult. Therefore, prophylaxis is an important issue in these patients.

Aims: We presented to use prophylactic rFVIIa in patient with severe Hemophilia-A with inhibitor.

Methods: Case: We present 13 years old boy with severe Hemophilia-A with inhibitor. His inhibitor titer was 170 Bethesda units (BU). He had developed target joint due to frequent bleeding under activated prothrombin complex concentrates (aPCC) prophylaxis given 50 unit kg⁻¹ every other day. His inhibitor titer increased from 170 BU to 220 BU in 6 months. During prophylaxis, he developed gross hematuria. Macroscopic hematuria intensively continued although additional doses of aPCC was given as 75 unit kg⁻¹ at 8 h intervals. His bleeding episode was taken under control with the administration of rFVII. It was initially given 90 µg kg⁻¹ at 2 h intervals for six times a day and then, macroscopic hematuria was resolved. The dose intervals decreased to 6 h. He had a well response to this treatment. Therefore, we decided to continue prophylaxis with rVIIa 90 µg kg day⁻¹ three times a week instead of aPCC.

Results: He has not suffered new joint bleeding or life threatening bleeding for 10 months under this new regimen. The inhibitor titer decreased from 220BU to 30.2BU after 4 months of rFVIIa prophylaxis.

Conclusion: rFVIIa is an effective drug for both controlling bleeding episodes and preventing new hemorrhages in hemophilia complicated with inhibitors. rFVIIa prophylaxis may provide safety from anamnestic responses until starting of immune tolerance treatment.

Disclosure of Interest: None declared.

PO157-TUE

Misfolded proteins in plasma-derived FVIII concentrates activate platelets and inhibit heme oxygenase-1 upregulation in macrophages by secreted platelet factor 4 (CXCL4)

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Background: Protein misfolding has been shown to induce platelet aggregation. Several FVIII products were found to contain misfolded proteins. A hallmark of hemophilia is hemorrhage into the joints. Heme mediates oxidative stress and inflammation leading to the destruction of cartilage and bone. The haptoglobin-CD163-heme oxygenase-1 (HO-1) pathway is a captor-receptor-enzyme system to evade heme-induced toxicity. Lately we showed that pdFVIII products, but not rFVIII, activate platelets, induce platelet microparticle formation and CXCL4 release.

Aims: As CXCL4 downregulates the protective hemoglobin receptor CD163 we studied the effect of rFVIII and pdFVIII products on macrophage differentiation.

Methods: Monocytes were isolated from fresh human blood and differentiated into macrophages in the presence of supernatant from platelets treated with factor VIII products (2 IU mL⁻¹). CD163 surface expression on macrophages and HO-1 expression were measured by flow cytometry. For assessment of HO-1 expression, macrophages were treated with autologous hemoglobin for 18 h.

Results: All of the eight tested pdFVIII products markedly induced CXCL4 release by platelets; whereas the four tested recombinant products had no such effect. Platelet activation was completely abolished after removal of misfolded proteins from pdFVIII products by affinity absorption, showing that CXCL4 secretion is due to misfolded proteins inside the pdFVIII products. Incubation with supernatants from pdFVIII treated platelets significantly reduced CD163 on macrophages and led to the production of macrophages that were unable to upregulate the joint protective enzyme HO-1 in response to hemoglobin.

Conclusion: PdFVIII products might boost blood induced joint injury via generation of macrophages that can not fight against ROS stress. Synovial macrophages from patients treated with either rFVIII or pdFVIII should be investigated.

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PO158-TUE

Circumcision in Turkish patients with bleeding disorders: a sociocultural perspective

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Background: Circumcision is one of the most commonly performed operations in children worldwide.

Aims: In this study, we evaluated thoughts about circumcision of the patients with bleeding disorders (BD) and their family.

Methods: We included 27 patients with BD whose average age were 16.3 ± 9.5 years. Evaluation questionnaire was administered to assess thought of patients and their family about circumcision developed by the study authors.

Results: In our study, 22 patients with bleeding disorders (81.4%) were circumcised. Twenty-four patients and their families (92.3%) consider that circumcision is a very important practice. Eleven of the patients and their families (47.8%) were very worried about circumcision due to risk of bleeding. In our study, we investigated the reasons for the request to be circumcised among the patients. Most of the families and patients (n = 25, 92.6%) consider that circumcision is a very important step toward being a man. Twenty two patients and their families (81.5%) think that circumcision must be performed because of religious necessity. Fifty-nine percent (n = 16) of patients and their families consider that being unable to be circumcised results in socially unacceptable and not to be viewed as a man. Additionally, Forty-eight percent (n = 13) of the study population believed that uncircumcised boy can not be married.

Although life-threatening effect for bleeding disorders, the majority of patients were circumcised. The present study, consistent with the literature, circumcision is performed as a social obligation for male patients with BD (2, 3, 4). The religious factors are the second line reason among patients with BD to get circumcised as a result of being a member of a Muslim society.

Conclusion: Among different nations, there are various reasons for circumcisions in BD. Cultural reasons for circumcision seems to be more dominant in our study population.

Disclosure of Interest: None declared.

PO159-TUE

Intra-laboratory variation in FVIII:C inhibitor assay: results of a study in three french centers

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Background: Detection and quantification of factor VIII (FVIII) inhibitors is clinically important for the management of Hemophilia A

treatment and acquired hemophilia diagnosis. Inhibitors assay protocol is complex; this leading to an important variation in results within- and between- all of the laboratories.

Aims: This study has objective to assess the degree of intra-laboratory variability by means of various protocols in three laboratories and to propose options to improve performances.

Methods: Three laboratories located in the same french area have been involved into such a study: Lyon, Grenoble, Saint-Etienne. Each of them have received six frozen plasma samples (0; 0.6; 1; 5; 10 and 70 UB mL⁻¹) which FVIII inhibitor level was early determined by referent Nijmegen method and prepared by CRYOPEP (Montpellier, France). Each laboratory tested 20 times the six samples for FVIII inhibitors by using their own protocol to assess the FVIII inhibitor level applying for Lyon and Saint-Etienne the modified Bethesda assay method whereas Grenoble the original one.

Results: No false-positive result found with sample 0 UB mL⁻¹. When all laboratories detect FVIII inhibitor in all other, the rate of false-negative results with 0.6 UB mL⁻¹ sample vary from 0% to 80%. Within Lyon laboratory, the minimum range CV_{intra} is 21.4% (10 UB mL⁻¹), the maximum 27.2% (70 UB mL⁻¹), their average is 24%. Within Saint-Etienne, the minimum range is 7.5% (1 UB mL⁻¹), the maximum 23.5% (0.6 UB mL⁻¹), their average is 15%. The best CV_{intra} average is 11%, within Grenoble laboratory, with a minimum range of 7.8% (10 UB mL⁻¹) and a maximum of 15.8% (5 UB mL⁻¹).

Conclusion: These results confirm an important within- and between-laboratory variations. Nonetheless Grenoble has the best variability explained by their low number of operators and by use of frozen reagents as FVIII source. One of the proposal to improve the repeatability of the FVIII inhibitor assay is therefore to decrease number of operators, to use frozen reagents and to repeat testing for confirmation.

Disclosure of Interest: None declared.

PO160-TUE

Stability of FVIII products – formation of subvisible particles upon physical stress

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Background: Subvisible particles (SVPs) in a size range of 0.1–10 µm were shown to facilitate unwanted immunogenicity of protein therapeutics. Their formation is a result of protein aggregation following exposure to various stress factors. Limited information is available on the formation of SVPs in FVIII products and their significance for the safety and functional properties of these products.

Aims: To assess the formation of SVPs in FVIII products upon exposure to physical stress.

Methods: Four commercially available recombinant FVIII (rFVIII) products (three lots each) were evaluated: two full-length (FL-) rFVIII products and two B-domain deleted (BDD-) rFVIII products. SVPs formation was assessed under conditions of intended use and after exposure to clinically relevant stress and to temperature stress (37 and 57 °C for 18 h). Samples were analyzed using a flow cytometry-based technology which facilitates the analysis of SVPs in the size range of 0.75–10 µm.

Results: Increased concentrations of SVPs were found in all rFVIII products after exposure to stress compared with conditions of intended use. FL-rFVIII products appeared to be more stable than BDD-rFVIII products, resulting in less SVP formation upon exposure to stress. Most dramatic differences in SVP concentration between FL-rFVIII and BDD rFVIII products (over 400-fold) were seen after exposure to extreme temperature stress (57 °C for 18 h). In addition, extreme temperature stress induced SVPs containing cross-beta-sheet structures in BDD-rFVIII but not in FL-rFVIII products.

Conclusion: FL-rFVIII and BDD-rFVIII products differ in their resistance to the formation of SVPs when exposed to clinically relevant or temperature stress. BDD-rFVIII products are more sensitive to stress, possibly due to their lack of most of the glycosylation sites on FL-rFVIII or to the reduced heterogeneity of the protein.

Disclosure of Interest: M. Malisauskas: None declared, C. Lubich Employee of: Baxter Innovations GmbH, T. Prenninger Employee of: Baxter Innovations GmbH, F. Scheifflinger Employee of: Baxter Innovations GmbH, B. Reipert Employee of: Baxter Innovations GmbH.

PO161-TUE

Comparison of FVIII activity of select novel recombinant FVIII replacement products in commonly used FDA approved one-stage clot assay systems

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Background: Reagent dependent recoveries for novel rFVIII replacement products have been described for FVIII one-stage (OS) clotting assays.

Aims: A side by side comparison of select novel rFVIII replacement products was performed, utilizing the most common FDA-approved, OS clot assay systems employed in the US. The percent recoveries of four novel rFVIII products were compared to two established rFVIII products, ADVATE® (Baxter International) and ReFacto® AF (Pfizer).

Methods: FVIII OS activity was determined for each product (based on labeled potency) at 80%, 30%, 15%, 5% and 1% activity spiked into hemophilia A plasma (HRF, Inc.). Samples were tested in triplicate following manufacturer recommended protocols, calibration and QC, on three separate occasions, in four FDA approved assay systems: STA-R Evolution® using PTT A and C.K. Prest® (Diagnostica Stago Inc.), BCS®XP using Dade® Actin® FSL (Siemens Healthcare) and ACL TOP® using HemosIL® SynthASil® (Instrumentation Laboratory).

Results: Linear recoveries for all rFVIII products were observed for three of the four assay systems. All six products over-recovered on the ACL TOP® at concentrations ≤15%. The four novel rFVIII products, across all concentrations tested, recovered within ±25% in at least one of the four assay systems. ReFacto® AF recovered within ±25% in two and ADVATE® in one of the assay systems, respectively.

Conclusion: The four novel rFVIII replacement products tested demonstrate accurate recovery within ±25% in at least one of the four commonly used, FDA approved, FVIII OS assay systems employed in the US. The STA-R Evolution® using PTT A or C.K. Prest® recovered the majority of the rFVIII products within acceptable limits. The assay system specific variability did not differ between novel and established rFVIII products. Thus the new generation rFVIII products show a similar assay system and product-specific recovery as already existed with currently approved products including ADVATE® and ReFacto® AF.

Disclosure of Interest: S. Tiefenbacher Consultant for: Novo Nordisk, M. Robinson: None declared, E. Ross: None declared, P. Williams: None declared, C. Cogswell: None declared, T. Wham: None declared, G. Chow: None declared, D. Adcock Consultant for: Novo Nordisk.

PO162-TUE

Investigating potential determinants of ITI success using immune gene expression profiling: a preliminary study

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Background: Immune tolerance induction (ITI) by prolonged administration of FVIII is currently the only proven strategy to eradicate FVIII inhibitors, although the success rate is no higher than 60–80%. Long-term FVIII-specific tolerance via ITI has been proposed to be the result of a complex, multifactorial process and the mechanisms underlying successful ITI have been unclear, and not much is known about the determinants of success vs. failure.

Aims: To assess the technical feasibility of a gene-expression profiling study in PBMCs from hemophilia A patients so to establish optimal experimental conditions (cell culture procedures, stimulations, and analysis timing) to develop a reliable means of pinpointing differences in immune response associated with ITI outcome in patients with hemophilia A and inhibitors.

Methods: Six severe hemophilia A patients who had developed high-titer inhibitors at the end of their first ITI were enrolled (age median [min–max] 12 [3–31], historical inhibitor peak of 88.5 [10–800] BU mL⁻¹, F8 mutation consisting of intron 22 inversion). Three of them were successfully tolerized while the remainder three patients experienced ITI failure. Fresh and cultured PBMCs – stimulated *in vitro* with recombinant and plasma-derived FVIII, and harvested at different time points – were used for RNA isolation.

Results: The results showed strong consistency across technical replicates. Data could be generated with as little as 250 ng of total RNA starting material, making it possible to work with a low number of cells for each condition. The 24 h time-point was associated with the strongest gene-expression modulation.

Conclusion: A comprehensive analysis of gene modulation could increase our knowledge of immune responsiveness to FVIII, clarifying which genes are up- or down-regulated, and which relevant immune pathways are involved in the process of ITI. A prospective study of gene-expression profiling is feasible and could help understand immunological mechanisms underlying success or failure.

Disclosure of Interest: None declared.

PO163-TUE

Factor VIII deficiency: inhibition or abnormal contact?

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Background: Initiation of the intrinsic pathway occurs when prekallikrein, high-molecular-weight kininogen, Factor XI (FXI) and FXII are exposed to a negatively charged surface, interacting with the phospholipids of circulating lipoprotein particles (contact phase). Autoantibodies bind FVIII in a time and temperature-dependent manner. Both situations influence aPTT and their clarification can be difficult.

Aims: Present the case of a patient with increased aPTT and FVIII low levels, with hematuria, whose diagnostic clarification has been very challenging.

Methods: Female, 22 years old, hospitalized for inaugural diabetes, secondary to corticoid therapy (prednisolone) prescribed 6 months before for migratory oligoarthritis. Sudden hematuria and aPTT ele-

vation (60–120 s), previous results border-line. Microcytic hypochromic anemia since three years ago, due to menometrorrhagia and *H. pylori* positive gastritis, Dress syndrome due to salazopyrine and acute hepatitis after ciprofloxacin treatment. Without previous transfusions or hemorrhagic discrasia. Family history of rheumatoid arthritis, absence of hemorrhagia. Preformed laboratorial investigation of aPTT elevation.

Results: FVIII activity levels average <5%, FVIII inhibitor positive (2.39 Bethesda units), FXII activity slightly decreased (44%), normal von Willebrand Factor and FXI. Lupus anticoagulant and Immunology study negative.

Medicated with cyclophosphamide and FVIII, with hematuria resolution, but aPTT elevation persistence.

Conclusion: Presence of border-line previous aPTT measurements, reinforces the hypothesis of an acquired abnormality.

Although hematuria has been solved, aPTT stills elevated, possible due to low FVIII levels associated with border-line FXII levels.

FVIII inhibitor levels are low and stable, apparently not influenced by immunosuppression and does not justify the severity of FVIII and aPTT results.

On the other hand, FVIII measurement himself can be influenced by FXII levels.

Other tests for contact phase study are still ongoing.

Disclosure of Interest: None declared.

PO164-TUE

***In vitro* factor VIII activity after reconstitution with diluent fluid for continuous infusion**

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Background: For the successful result one's blood coagulation factor level during and after surgery is important. Continuous infusion method has merit of saving product as much as 30%. Most physicians reconstitute the lyophilized powder with diluent for 24 h amounts at a same time. Because the drug activity will be decreased gradually after dilution even *in vitro* the latter portion of infusion will have decreased activity also.

Aims: We evaluate *in vitro* factor VIII activity after reconstitution with diluent fluid for continuous infusion in case of surgery with hemophilia A patients.

Methods: We checked the *in vitro* activities on 0, 8, 16 and 24 h after dilution with one commercial plasma derived FVIII product. And three commercial FVIII products (2 recombinant FVIII and 1 plasma derived concentrates) were used for *in vitro* FVIII:C activities at 0, 2, 4, 6, and 8 h after reconstitution in both the exposure to indoor light group and the light shield group.

Results: *In vitro* activity was gradually decreased to $91 \pm 5.3\%$, $80 \pm 6.9\%$, and $56 \pm 6.7\%$ at 8, 16 and 24 h on room temperature and on room light exposure respectively. Better results of $94 \pm 4.8\%$, $89 \pm 6.5\%$ and $73 \pm 6.4\%$ on light shield were observed ($P < 0.001$). In the three drugs, *in vitro* FVIII:C was decreased from 0 to 8 h after reconstitution ($P < 0.001$). The decline of FVIII:C followed linear model ($P < 0.001$). *In vitro* FVIII:C was $95.3 \pm 1.91\%$ and $90.6 \pm 2.53\%$ at 4 and 8 h after reconstitution, respectively, in exposure to room light group. On light shield group, FVIII:C was

$95.4 \pm 1.12\%$ and $90.9 \pm 1.67\%$ at 4 and 8 h, respectively ($P = 0.849$).

Conclusion: We aware the longer *in vitro* dilution state the lower factor activity. So, we have to adjust the required amount according to the frequency of changing bottle for the best bleeding control during and after surgery. Reconstitution of lyophilized powder with diluent every 4 h is desirable to maintain effective blood factor level.

Disclosure of Interest: None declared.

PO165-TUE

Application of clot waveform analysis on hemostatic monitoring for factor (F)VIII mimicking anti-FIXa/FX bispecific antibody (ACE910)

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Background: ACE910, bispecific monoclonal antibodies to FIXa and FX, mimics the FVIIIa cofactor function, and improves aPTT in FVIII-deficient plasma (FVIII_{def}) by substituting for FVIIIa. aPTT overestimates coagulation potentials however, resulting in not reflecting FVIII:C. Clot waveform analysis (CWA) is useful to assess global clotting function.

Aims: We attempted to establish the hemostatic monitoring for ACE910 using CWA.

Methods: CWA was performed on CS-2000i® (Sysmex), and clot time (CT), maximum coagulation velocity and acceleration (|min1|, |min2|) were monitored. Elg and Elg/TF were used as trigger.

Results: ACE910 markedly improved all parameters in FVIII_{def} and FVIII_{def} with 10 BU mL⁻¹ anti-FVIII IgG (FVIII_{IgG}). ACE910 30 µg mL⁻¹ corresponded to FVIII relative activity of 6–13 IU dL⁻¹ in a primate acquired model (JTH, 2014), and ACE910 10–100 µg mL⁻¹ is considered as prophylactic range estimated from first clinical trial (Shima, ASH2014). Since prevention of the joint bleeding in HA requires at least FVIII:C 12 IU dL⁻¹ (FVIII₁₂), FVIII_{def} mixed with rFVIII (12 IU dL⁻¹) were prepared. Elg-triggered CT in FVIII_{def} or FVIII_{IgG} with even ACE910 0.3 µg mL⁻¹ was markedly shortened ($44 \pm 1/44 \pm 2$ s) beyond normal levels, similar to that in FVIII₁₂ (43 ± 1), and Elg-triggered |min1|/|min2| were similar level among in FVIII_{def} ($3.9 \pm 0.2/0.5 \pm 0.1$), in FVIII_{IgG} with ACE910 10 µg mL⁻¹ ($3.7 \pm 0.1/0.5 \pm 0.1$) and in FVIII₁₂ ($3.6 \pm 0.1/0.4 \pm 0.1$), indicating overestimated relative-FVIII:C. The similar experiments with Elg/TF trigger were performed. CWA revealed that the CT in FVIII_{def} and FVIII_{IgG} with ACE910 30 µg mL⁻¹ were similar to that in FVIII₁₂ (111 ± 7 , 102 ± 14 , 111 ± 3 s). |min1| and |min2| in FVIII_{def} and FVIII_{IgG} with ACE910 30 µg mL⁻¹ also showed similar levels that in FVIII₁₂ ($1.0 \pm 0.8/0.06 \pm 0.01$, $1.1 \pm 0.3/0.08 \pm 0.06$, $1.2 \pm 0.1/0.05 \pm 0.01$), supportive of better trigger of Elg/TF in CWA.

Conclusion: CWA using mixed trigger with Elg/TF is a promising method for the hemostatic monitoring of ACE910 therapy.

Disclosure of Interest: T. Matsumoto Grant/Research Support from: Chugai Pharmaceutical Co.,Ltd., K. Nogami Grant/Research Support from: Chugai Pharmaceutical Co.,Ltd., Consultant for: Chugai Pharmaceutical Co.,Ltd. and F. Hoffmann-La Roche Ltd., K. Yada Grant/Research Support from: Chugai Pharmaceutical Co.,Ltd., T. Kitazawa Shareholder of: Chugai Pharmaceutical Co.,Ltd., Employee of: Chugai Pharmaceutical Co.,Ltd., K. Hattori Shareholder of: Chugai Pharmaceutical Co.,Ltd., Employee of: Chugai Pharmaceutical Co.,Ltd., M. Shima Grant/Research Support from: Chugai Pharmaceutical Co.,Ltd., Consultant for: Chugai Pharmaceutical Co.,Ltd.

Contact pathway

PO166-TUE

Efficacy of a novel contact pathway inhibitor, IR-CPI, in an extracorporeal membrane oxygenator model

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Background: Ir-CPI, a protein derived from the tick *Ixodes ricinus* salivary, is a serine protease inhibitor of both FXIIa and FXIa. Heparins remain suboptimal in managing bleeding complications when extracorporeal life support is required.

Aims: The aim of this study was to evaluate whether inhibition of the contact phase of the coagulation cascade might confer antithrombotic activity.

Methods: Twelve Beagle dogs were included. A pediatric ECMO system was connected between the carotid artery and the jugular vein, and a cardiopulmonary bypass was maintained for 90 min at the level of full-flow systemic perfusion. Anticoagulation was performed by UFH (300 IU kg⁻¹) with protamine reversal at the end of the procedure ($N = 3$). Three investigative groups ($3 \times N = 3$) received various doses of Ir-CPI (bolus and infusion) total dose 4.5 to 24 mg kg⁻¹. After disconnection, circuits and cannulae were examined for clot and protein deposits. All animals were followed-up and sampled for determination of Ir-CPI plasma concentration (Mass Spectrometry), aPTT, FXI and FXII residual activities, before necropsy on Day 7.

Results: All 12 animals completed procedure. First Ir-CPI group showed fibrin deposits, increased pressure gradient in the circuit, and subsequent decreased gas exchanges. In further animals perfusion of Ir-CPI was initiated earlier in order to decrease the observed clot initiation and to improve gas exchanges. High dose achieved close to optimal control. Low dose was less satisfactory but perfusion remained possible. A dose-dependent and related increase in Ir-CPI plasma concentration, increase in aPTT ratio and inhibition of factors XI and XII (20% and >70% respectively) were measured over the course of the procedure. No side effects were observed and necropsies confirm the absence of thrombosis or hemorrhage.

Conclusion: Ir-CPI can be used as a single agent to inhibit the intrinsic coagulation pathway and achieve anticoagulation. Efficiency can be improved without increasing bleeding risk.

Disclosure of Interest: S. Combe Shareholder of: the company developing agent, Consultant for: the company developing agent, Y. Fromes Consultant for: the company developing agent, C. Krezel Consultant for: the company developing agent, P. Gueret Grant/Research Support from: the company developing agent, J. Amiral: None declared, M. Guyaux Shareholder of: the company developing agent, Consultant for: the company developing agent, E. Godfroid Shareholder of: the company developing agent, Employee of: the company developing agent.

PO167-TUE

Most of Lupus anticoagulant in recurrent pregnancy loss patients recognize high molecular weight kininogen and/or factor XII

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Background: Lupus anticoagulant (LA) detection should be performed according to the guidelines by the International Society on Thrombo-

sis and Haemostasis – Scientific and Standardization Committee (J Thromb Haemost 2009; 7: 1737–40). One of the important recommendation in ISTH-SSC guidelines is to perform two different tests that represent different assay principles. There is an evidence that no single test is sensitive for all LA. dRVVT should be the first test considered. The second test should be a LA sensitive aPTT. Any aPTT test performed with silica as an activator and low phospholipids content is the second test of choice because of its sensitivity for LA.

Aims: We examined the association between LA sensitive aPTT and autoantibodies to high molecular weight kininogen (HK) and factor XII (FXII).

Methods: Recurrent pregnancy loss (RPL) patients ($n = 529$) were screened for aPTT mixing test, dRVVT, kininogen-dependent antiphosphatidylethanolamine antibodies (aPE), anticardiolipin antibodies (aCL) and FXII activities. In this present study, research ethics committee approval and patient written informed consents were obtained.

Results: Among 48 patients (9.1%) who were positive for aPTT mixing test (ICA>7), 10 patients (20.8%) were positive for aPE, 34 patients (70.8%) were deficient for FXII (<60%) and 38 patients (79.2%) were aPE positive and/or FXII deficient. Unlike the dRVVT which stimulates factor X, aPTT test can detect autoantibodies to contact proteins such as high molecular weight kininogen (HK) and FXII which are upstream from factor X. Among 5 patients (0.9%) who were positive for dRVVT (>1.2), no patients were positive for aPE, no patients were deficient for FXII. No patients were positive for both aPTT and dRVVT.

Conclusion: Our present data suggest that most of LA in RPL patients recognized HK and/or FXII.

Disclosure of Interest: None declared.

PO168-TUE

Factor XIA, factor IXA and tissue factor activity in blunt trauma patients

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Background: An elevated procoagulant activity has been observed in trauma patients. In part, this increased activity is related to tissue factor (TF) located on blood cells and microparticles. However, quantitated levels of TF in trauma patient plasma indicate that there are other contributor(s) to the procoagulant activity. We hypothesize that factor (F)XIa and FIXa are responsible for an additional activity in blunt trauma patients.

Aims: To analyze plasma from blunt trauma patients for the presence of FXIa, FIXa and TF and quantitate their concentrations.

Methods: Multiple time-point citrate plasma samples from 53 blunt trauma patients (total number of samples analyzed was 180; up to 11 time-point samples per patient collected in 120 h after admission) were evaluated in a thrombin generation assay using anti-FXIa, anti-FIXa and anti-TF monoclonal inhibitory antibodies. Forty-one patients were male 12 female. The age of patients varied between 19 and 92 years. The Injury Severity Score varied between 1 and 75.

Results: At the time of admission, 22 patients (42%) had active TF, 50 (94%) had FXIa and 9 (17%) had FIXa in their plasma. Nine patients (17%) without TF activity at the admission showed that activity at later time-points. As for FXIa and FIXa, 100% of patients had FXIa and 30% had FIXa at at least one time-point. The concentration of TF was relatively low and varied between 0.1 and 1.5 pM, whereas that of FXIa varied between 0.5 and 10 pM for the majority of plasma samples, but in some samples exceeded 100 pM. Plasma samples with FIXa activity had that protein at sub-nanomolar concentrations. No TF, FXIa and FIXa activity was detected in plasma from healthy individuals.

Conclusion: The majority of plasma samples from blunt trauma patients have detectable FXIa activity, with significant fraction of

them having active TF and FIXa as well. The concentration of all three proteins in patient plasma varies in a wide range.

Disclosure of Interest: None declared.

PO169-TUE

Coagulation monitoring of ventricular assist device recipients reveals new insights in disturbed haemostasis

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Background: Patients with advanced heart failure far outnumber the hearts available for transplantation. Partly as a consequence of this shortage, ventricular assist devices (VADs) are being used more widely. Coagulation monitoring is a central component of recipient's management because thromboembolism and bleeding are strongly associated clinical outcomes. Studies on platelet function have shown that after VAD insertion, an up-regulation of platelet activation markers and function occurs. Furthermore, the recipient's shows reduced high molecular weight von Willebrand factor multimers (acquired von Willebrand syndrome).

Aims: To date scientific findings on regulatory mechanisms of the coagulation system in VAD recipients are rare. The present study aimed to investigate the course of clot formation properties in VAD recipients in intensive care unit (ICU). Furthermore, the study reveals a more detailed view of the disturbed haemostasis and its correlation with bleeding and thromboembolism events.

Methods: Thrombin generation is measured in platelet-poor (PPP) and platelet rich plasma (PRP). Platelet function is assessed as P-selectin expression after platelet activation and in Thromboelastography as Platelet Mapping. Spatial propagation of the clot is recorded using the Thrombodynamics assay. Furthermore, classical coagulation parameters were determined. Patients are monitored on daily basis before and after implantation of a VAD.

Results: Will be included later.

Conclusion: Will be included later.

Disclosure of Interest: None declared.

PO170-TUE

Histidine-rich glycoprotein binds DNA and RNA and attenuates their capacity to activate the intrinsic pathway of coagulation

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Background: DNA and RNA are physiological activators of the contact system of coagulation. Histidine-rich glycoprotein (HRG) modulates the contact system by binding factor (F) XIIa and attenuating its activity.

Aims: To identify the mechanisms by which nucleic acids promote contact activation, and to determine how HRG modulates this process.

Methods: Thrombin generation and clotting assays were initiated with DNA or RNA in normal or HRG-deficient human plasma. The effect of HRG on DNA- or RNA-mediated activation of FXII and FXI in

the absence or presence of contact factors and thrombin was assessed using chromogenic assays, while the binding of contact proteins and thrombin to immobilized DNA or RNA was assessed using surface plasmon resonance (SPR).

Results: Addition of DNA or RNA to human plasma enhanced thrombin generation via the intrinsic pathway and shortened the clotting time. Their effect on the clotting time was 7- to 14-fold greater in HRG-deficient plasma than in control plasma; therefore, HRG attenuates nucleic acid-induced activation of coagulation. Investigations into the mechanisms of activation revealed that nucleic acids (a) promoted FXII activation in a prekallikrein (PK)- and high molecular weight kininogen (HK)-dependent fashion, and (b) enhanced FXI activation by thrombin by 10- to 12-fold. SPR studies showed that HRG binds DNA and RNA with K_d of ~ 1 nM, and functional studies revealed that HRG attenuates DNA- and RNA-mediated FXII activation, and FXI activation by FXIIa or by thrombin. SPR studies demonstrated that FXII, FXIIa, HK, FXI, FXIa and thrombin also bind DNA and RNA, suggesting that nucleic acids provide a surface onto which numerous contact factors assemble to trigger coagulation. Therefore, in addition to attenuating FXIIa activity, our findings suggest that HRG down regulates the capacity of nucleic acids to initiate and propagate the intrinsic pathway of coagulation.

Conclusion: By binding nucleic acids and FXIIa, HRG is a novel modulator of the intrinsic pathway of coagulation.

Disclosure of Interest: None declared.

PO171-TUE

Localization of factor XI in platelets is not restricted to alpha granules

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Background: Thrombin activation of platelets on a fibrinogen surface results in two stages of shape changes. First, platelets develop finger-like projections from cell periphery and granules are squeezed into the center of the cell. Next, platelets flatten into a fried egg appearance. The presence of factor XI protein (FXI) in platelets is controversial. Factor XI-like antigenicity and activity were shown in platelets. Interestingly, platelet FXI displayed a different size than the plasmatic one. Moreover, FXI mRNA was also presented in platelets but whether this mRNA is translated into protein is yet to be determined.

Aims: To confirm the presence of FXI in platelets and investigate its localization using confocal immunofluorescence.

Methods: Washed platelets were placed on fibrinogen-coated glass cover slips and probed for FXI, actin and p-selectin, as a marker of alpha granules, using specific antibodies. Platelets were allowed to adhere and spread for 10 min at 37 °C. Then thrombin was added and incubated for 2 or 40 min. Following thrombin activation samples were fixed and permeabilized.

Results: Confocal immunofluorescence staining confirmed the presence of FXI protein within platelets. After two minutes of platelet activation by thrombin, platelets exhibited fingerlike projections and both FXI and p-selectin were displayed at the center of platelets, but only partial co-localization was demonstrated. After 40 min of thrombin activation, platelets exhibited fried egg appearance, FXI staining declined and was observed as small dots scattered differently from p-selectin and from the 2-min activation presentation.

Conclusion: We confirm the presence of FXI protein within platelets. Its localization is not restricted to alpha granules. Upon activation, the localization of platelet FXI changes and decrease, probably due to secretion. FXI that is secreted from platelets following their activation could be involved in thrombus formation.

Disclosure of Interest: None declared.

PO172-TUE

The 2,5-dichlorophenyl fragment as starting point in the design of new FXIIa inhibitors

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Background: Thrombotic diseases are a major health concern. Anticoagulants have proven their efficacy but they are still associated with bleedings. In the quest for the ideal anticoagulant, the inhibition of FXIIa emerges as an attractive strategy for the development of safe antithrombotic drugs.

Aims: Coumarin derivatives have been previously described as small-molecular-weight inhibitors of FXIIa. Unfortunately, these compounds did not display any activity *in vivo* in a model of thrombosis. In order to develop a new chemical scaffold targeting FXIIa, we launched a study derived from a fragment-based drug design approach. This study aimed to evaluate the fragment expansion of a 2,5-dichlorophenyl moiety. This latter was selected upon the assumption that 1) thrombin and FXIIa should share structural features since they belong to the trypsin-like serine protease family and 2) the 2,5-dichlorophenyl moiety is present in various thrombin inhibitors and is found to be stabilized in the thrombin S1-pocket as demonstrated by crystallographic studies.

Methods: About twenty compounds containing a 2,5-dichlorobenzyltriazole scaffold were obtained by *click* chemistry. Then, from the most active compounds, we investigated the replacement of the 2,5-dichlorophenyl by other halogens. Their inhibition potency was then measured on FXIIa.

Results: Six compounds from the first series were selective and displayed IC₅₀ ranging from 10 to 100 μ M. Then, we studied the replacement of the 2,5-dichlorophenyl moiety. This underlined the importance of the 2,5-dichlorophenyl moiety in the FXIIa inhibition. This is an important information for our next syntheses.

Conclusion: The results are really encouraging and novel syntheses have been started. They include the evolution of the side chain on the 2,5-dichlorobenzyltriazole. Molecular modeling of these compounds will be helpful to rationalize the design of new attractive FXIIa inhibitors. Investigation of these new molecules on thrombin generation assay is also in the pipeline.

Disclosure of Interest: None declared.

PO173-TUE

DSR-130787, a novel orally-active factor XIA inhibitor with low risk of bleeding

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Background: Factor XI (FXI) deficiency causes hemophilia C, which is characterized by mild spontaneous bleeding. As patients with severe FXI deficiency have been reported to be at risk of ischemic stroke, FXIa is considered to be an attractive target for the development of novel anticoagulants. We have recently found DSR-130787 as a novel orally-active prodrug FXIa inhibitor.

Aims: The aim of this study is to evaluate the pharmacological profiles of DSR-130787 and its active form.

Methods: First, the inhibitory effects of test-compounds on human FXIa, as well as on other serine proteases involved in coagulation or fibrinolysis were evaluated. The effects of the active form of DSR-130787 on thrombosis and hemostasis were then compared to those of

dabigatran and rivaroxaban in rabbit models of arteriovenous-shunt thrombosis and cuticle bleeding time. Finally, the anticoagulant effect of single oral administration of DSR-130787 was evaluated in cynomolgus monkeys.

Results: The active form of DSR-130787 potently inhibited human FXIa ($IC_{50} < 10$ nM) with a selectivity approximately 1000-fold that for other serine proteases. DSR-130787 active form concentration-dependently prolonged aPTT in human, rabbit and monkey plasmas [EC_{2x} values of 0.58, 36, and 2.7 μ M, respectively]. In rabbits, intravenous administration of DSR-130787 active form produced a dose-dependent antithrombotic effect comparable to that of dabigatran or rivaroxaban with minimal change in bleeding time. In monkeys, the prolonging effect of DSR-130787, given orally at 30 mg kg^{-1} , on aPTT lasted for up to 24 h.

Conclusion: DSR-130787 active form potently and selectively inhibited human FXIa. Intravenous administration of DSR-130787 active form to rabbits produced an antithrombotic effect comparable to that of dabigatran or rivaroxaban with much lower risk of bleeding. DSR-130787 is therefore expected to be a novel orally-active anticoagulant with low bleeding risk.

Disclosure of Interest: None declared.

PO174-TUE

Evaluation of the effects of a novel contact pathway inhibitor, Ir-CPI, on *in vitro* platelet function and coagulation

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Background: Ir-CPI, the tick *Ixodes ricinus* salivary protein, is a serine protease inhibitor under development as a novel anticoagulant. Ir-CPI inhibits Factors (F) XIIa, XIa, and kallikrein generation, prolongs the activated partial thromboplastin time, and confers antithrombotic activity in preclinical models.

Aims: The study aim was to test Ir-CPI effects on platelet function using light transmission aggregometry (LTA), clot retraction, and P-selectin expression and to characterize Ir-CPI inhibition of the contact activated coagulation pathway by assessing FXa and FIXa activation time and activity.

Methods: Ir-CPI effects on LTA in response to 20 μ M ADP and 1.6 mM arachidonic acid (AA) were studied using consented blood donors ($n = 5$). Platelet P-selectin expression was evaluated using flow cytometry ($n = 4$) following activation by contact pathway initiation. Ir-CPI effects on PRP clot retraction was measured ($n = 5$), and FXa and FIXa generation were determined by quantitative chromogenic assays ($n = 3$).

Results: LTA response as well as clot retraction were unaffected by Ir-CPI pretreatment. However, the percent of P-selectin positive platelets was decreased following Ir-CPI treatment (0.5, 1, and 2 μ M) by 5%, 9%, and 18%, respectively, vs. control. FXa activation lag times were prolonged to 704, 752, 826, 1013 s with increasing concentrations of Ir-CPI (1, 2, 4, 8 μ M, respectively) vs. control (611 s). Similarly, FIXa activation lag times were 790, 834, 876, and 902 s, respectively, vs. 581 s (control). Also, the mean reaction rates (OD min^{-1}) of FXa and FIXa with added Ir-CPI were decreased ~ 2.3 -fold.

Conclusion: We demonstrated that Ir-CPI does not affect *in vitro* platelet aggregation response or clot retraction. However, Ir-CPI dose dependently blocked contact pathway mediated platelet P-selectin expression and the rate of FXa and FIXa activation and activity. This study confirms specific effects of Ir-CPI on the contact pathway of coagulation and provides a basis for monitoring Ir-CPI.

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PO175-TUE

Blood group, bleeding phenotype and post-partum haemorrhage in factor XI-deficient women

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Background: Factor XI (FXI) deficiency is a rare bleeding disorder with an autosomal dominant inheritance. Heterozygotes have a mild/moderate reduction in FXI level (20–70 iu dL^{-1}), and homozygotes or compound heterozygotes have severe reduction in levels, often <1 iu dL^{-1} . However, there is no clear correlation between the FXI level and bleeding tendency. Bleeding is usually trauma or surgery-related. The variable bleeding tendency and risks of factor replacement pose a challenge to pregnancy-management.

Aims: Our aim in this study was to examine the post-partum haemorrhage (PPH) rate in women with FXI deficiency, to enable better risk-stratification at delivery.

Methods: FXI deficient-women were identified from local data-bases of 5 hospital trusts. Those who had at least one pregnancy were identified from obstetric records. Subjects were divided into 'bleeders' and 'non-bleeders' according to their general bleeding tendency. Blood group, baseline FXI level, PPH events and vonWillebrand antigen and activity were all recorded where available.

Results: A total of 115 women were identified. Overall PPH rate in FXI-deficient women was 16.2% compared to local PPH rates of 5%. Of these, PPHs occurred in 21.5% 'bleeders' compared to 10% in non-bleeders, and 26.4% of blood group O compared to 8% in Non-O. Women with combined blood O and bleeding phenotype had the highest PPH rate of 32.8%. Mean FXI level was similar across all subgroups. Von Willebrand factor antigen and activity results were available for 35 women.

Conclusion: Although the majority of women with FXI deficiency have straightforward pregnancy and delivery, those with a history of bleeding and/or blood group O may experience excessive bleeding at or after delivery. Expectant management with availability of blood products should be practiced for these women.

Disclosure of Interest: None declared.

PO176-TUE

Identification of three novel factor XII mutations associated with congenital factor XII deficiency

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Background: Congenital factor XII (FXII) deficiency is a rare, autosomal recessive coagulation disease caused by mutations in the *F12* gene. We have diagnosed five Chinese pedigrees with FXII deficiency.

Aims: To investigate the underlying mechanism of the FXII deficiency phenotype in these pedigrees.

Methods: FXII antigen and FXII activity levels were determined by ELISA and one-stage clotting assays, respectively. Mutations in *F12* gene were analyzed by sequencing the entire exons. Mutations were introduced into a pIRES2-EGFP-FXII mammalian expression plasmid by site-directed mutagenesis, and wild-type or mutant FXII proteins were expressed transiently in HEK 293T cells. Protein degradation inhibition experiments were performed by using various membrane-permeable inhibitors.

Results: All patients had prolonged activated partial thromboplastin time, as well as markedly decreased FXII activity and antigen level. Gene analysis identified three mutations [c.6635G>A (p. G259E), 6658G>C (p. R267G) and c.8489G>A (p. E521K)] in the five patients, two of whom were with heterozygous mutations (R267RG and E521EK, respectively). *In vitro* studies in transiently transfected HEK 293T cells demonstrated that these mutations significantly lowered the FXII levels in the culture media, but not due to the defects in transcription or transportation through the secretion pathway. Protein degradation inhibition experiments with various inhibitors suggested that the three mutants were degraded intracellularly through the proteasome pathway in the pre-Golgi compartment. Moreover, R267G and G259E mutations exhibited dominant negative effects leading to FXII deficiency, consistent with the phenotypes in the heterozygous carriers, but such dominant negative effects were not due to the dimerization of FXII.

Conclusion: In summary, we have identified and characterized three novel missense mutations that caused FXII deficiency in five patients, with one patient exhibiting autosomal dominant trait.

Disclosure of Interest: None declared.

PO177-TUE

FXI-independent cerebral hemostasis in experimental traumatic brain injury

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Background: Polytrauma victims are at high risk of injury-associated bleeding and thrombosis. Antithrombotic treatment benefits patients,

but concurrent traumatic brain injury (TBI) represents an important medical dilemma due to high risk of increased hemorrhage. The brain expresses high levels of tissue factor (TF) that supports hemostatic intracranial thrombin generation. While microthrombi likely control head injury-associated intracranial bleeding, they may also play a role in TBI-associated cerebral ischemia. Because antithrombotics inhibit TF-dependent hemostasis the risks of their use in TBI is considered to outweigh the antithrombotic benefits.

Aims: Unlike TF-dependent hemostasis, contact pathway activation does not appear to support hemostasis and inhibition of contact pathway factors XII or XI are antithrombotic. Here we evaluated whether inhibition of contact pathway activation affects intracranial bleeding and microthrombus formation in a mouse model of TBI.

Methods: Wild type mice received an antibody (14E11) that inhibits FXI activation by FXIIa or a control IgG 24 h before, 30 min or 2 h after TBI. Pial and parenchymal microvessels were visualized by 2-photon microscopy 2–3 h after trauma and blood vessel occlusions and cortical perfusion were evaluated. Histopathological outcome, including bleeding, microclots, and edema, was quantified 24 h after TBI.

Results: Both pharmacologic inhibition of FXI activation by FXII and genetic deletion of FXI prolonged aPTT without affecting PT. This anticoagulation that targets FXII and FXI dependent thrombin generation was not associated with changes in intracranial hemorrhage, microthrombus formation, or hemispheric swelling within the first 24 h following TBI.

Conclusion: Since thrombogenesis in larger vessels is FXI-dependent, multitrauma victims might benefit from pharmacologic targeting of FXI-dependent thrombin generation and thrombosis without aggravating TBI-associated brain hemorrhage.

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PO178-TUE

Activation of factor XII by plasmin leads to bradykinin formation in hereditary angioedema

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Background: Patients with hereditary angioedema (HAE) develop bradykinin-mediated focal attacks of pathological vascular leakage at various sites of the body. HAE is caused by C1 esterase inhibitor deficiency (HAE-C1inh) or by substitution mutations in factor XII (HAE-FXII). These mutations include T309K, T309R, or a mutation that replaces 16 original amino acids by 27 new amino acids (Del&Ins).

Aims: To elucidate the disease mechanism of HAE-FXII.

Methods: *In silico* predictions indicate that HAE-FXII mutations induce loss of O-linked glycosylation and simultaneously introduce new enzymatic cleavage sites for trypsin-like proteases. We produced recombinant mutants and wild type FXII for comparative studies. Control mutants T309A and 'Del' (only lacking O-linked glycosylation) were made to investigate the influence of both types of molecular changes.

Results: HAE-mutations did not enhance clotting- or enzymatic activity on a kaolin surface. However, FXII can also be activated through cleavage by plasma kallikrein (PK) and plasmin in solution. We found

that plasmin, but not PK, cleaved all HAE-FXII mutants while leaving controls intact. Corresponding to this cleavage, enzymatic activity increased dramatically.

In plasma, HAE-FXII mutants escaped inactivation by Clinh in response to plasmin. The resultant free activated FXII generated a strong pulse of bradykinin release via PK. Soluble lysine analogues attenuated this mode of FXII activation, explaining their reported therapeutic effect in HAE. These findings were confirmed in plasma of patients with FXII-T309K. Disease activity correlated positively with plasminogen levels in these patients, suggesting that it may act as a disease modifier.

Conclusion: HAE-FXII mutations collectively introduce new cleavage sites in FXII that accelerate activation by plasmin, leading to excessive bradykinin release. We propose that this implicates plasmin as trigger for FXII activation in HAE and hypothesize that this mechanism reflects the natural pathway by which FXII is activated.

Disclosure of Interest: None declared.

PO179-TUE

Unexpected hepatocyte growth factor activator (HGFA) activity allows a possible crosstalk between activated factor XII and the HGF/c-MET pathway in human plasma

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Background: Hepatocyte growth factor (HGF) promotes cell proliferation/angiogenesis through its c-Met receptor. Hyperactivation of c-MET highly express transcription of genes such as that corresponding to plasminogen activator inhibitor 1 (PAI-1) and cyclooxygenase 2 (COX-2), promoting hemostasis activation and fibrin deposition. Factor XII (FXII) may not be essential for coagulation *in vivo*, but promotes cell proliferation/angiogenesis and thrombosis. HGF activator (HGFA) is a FXII-like serine protease and chicken genome presents its corresponding gene, but not that of FXII. HGFA, known as thrombin substrate, is the main activator of HGF.

Aims: Our objective was to detect possible activation of HGFA after treatment of citrated human and chicken plasma samples with ellagic acid (EA)-based reagent.

Methods: The hydrolysis of the fluorogenic substrate Ac-K-T-K-Q-L-R-MCA designed for HGFA was monitored in a spectrofluorimeter under different experimental conditions.

Results: In human citrated plasma, EA was able to promote cleavage of synthetic substrates designed for active FXII (FXIIa), plasma kallikrein (KK), active factor XI (FXIa) and, surprisingly, active HGFA. However, no activity concerning HGFA substrate was observed in similar conditions with chicken or FXII- and PPK-deficient human plasma samples under the same experimental conditions.

Conclusion: The cleavage of HGFA substrate was dependent on FXIIa and KK. FXIIa itself and other FXII-driven proteases, such as FXIa, KK, KK-activated urokinase (uPA) and bradykinin-released tissue-type plasminogen activator (tPA) can alternatively activate HGF *in vitro*. The use of specific antibodies or inhibitors for c-MET receptor and/or its intracellular signaling pathways would elucidate possible influence of HGF/c-MET pathway in both cell proliferation/angiogenesis and thrombus generation elicited by FXII.

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PO180-TUE

FXI and FXII activity levels in young patients with cryptogenic ischemic stroke

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Background: Experiments with Factor XII (FXII) knockout mice have demonstrated that fibrin formation mediated by FXII and FXI is important for pathological arterial thrombosis. These studies have also showed that FXII deficiency prevents pathological thrombus formation without affecting normal haemostasis. However, it is unknown if FXII and FXI contribute to stroke pathophysiology in humans.

Aims: The aim of this study is to determine FXI and FXII activity levels in young people with stroke.

Methods: Population: Stroke Group (SG): 37 consecutive patients (22–55 years old) with cryptogenic arterial cerebrovascular disease confirmed by image studies were included between 2010 and 2014 as part of a Multicenter National Study for the detection of Fabry disease in young people (AISYF trial). A CT scan, MRI, carotid ultrasonography and transthoracic echocardiography and EKG monitoring was performed in all patients. 15/37 present at least one classical cardiovascular risk factor and all had a negative thrombophilia study. Mean NIHSS score was 4 (1–7). Control group (CG): 70 healthy controls. Method: FXI and FXII were performed in three dilutions by one stage clotting assay in automated coagulometer.

Results: Stroke group mean age was 41.9 years old (range: 22–55), 22 women; Control group mean age was 38 years old (25–50), 18 women. FXI activity levels were SG: $121.12 \pm 28.32\%$ and CG: $101.74 \pm 12.58\%$. Three patients presented FXI levels above 90 th percentile of control group. FXII activity levels were: SG: $116.57 \pm 34.83\%$ and CG: $102 \pm 18.32\%$. Seven patients showed FXII levels above 90th percentile of control group. In this study, neither FXI nor FXII activity levels in the stroke group were significantly different from the control group.

Conclusion: According with these results, FXI and FXII activity levels doesn't appear to be associated with cryptogenic ischemic stroke.

Disclosure of Interest: None declared.

PO181-TUE

Antithrombotic effects of Ir-CPI in an arterio-venous shunt model in the rabbit

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Background: Ir-CPI is a 67 aa protein derived from the salivary glands of the tick *Ixodes ricinus*. It is a contact phase inhibitor targeting specifically factors XIa and XIIa.

Aims: The aim of the study was to evaluate the antithrombotic potential and the pharmacokinetic-pharmacodynamic relationships of Ir-CPI.

Methods: The experiment was performed on New Zealand rabbits ($n = 34$) anesthetized with ketamine-xylazine. An arterio-venous (AV) shunt device containing a silk thread was connected between the femoral artery and vein. Ir-CPI was administered intravenously at variable doses as a bolus alone or followed by a continuous infusion starting 5 min before the opening of the AV-shunt. Thrombus weight was measured at the end of a 40 min shunt period. Blood samples were taken 10 min before and 45 min after administration. Plasma was prepared

to monitor the activated partial thromboplastin time (aPTT), FXI and FXII activities and Ir-CPI concentration. The aPTT was measured using actin FS as reagent. Factors XI and XII activities were measured using FXI or FXII deficient plasmas and aPTT (Cephen) method. Ir-CPI plasma concentration was determined by mass spectrometry and enzyme-linked immunosorbent assay.

Results: Ir-CPI reduced thrombus weight by 36.8%, 62.1% and 97.2% at 1, 3 and 5 mg kg⁻¹, iv and by 90.4% at 3 mg kg⁻¹ followed by 2.3 mg kg⁻¹ h infusion. Ir-CPI plasma concentrations increased proportionally with the dose. Antithrombotic activity (>90%) corresponded to a plasma Ir-CPI level of 2500 ng mL⁻¹, a prolongation of the aPTT of 50%, an inhibition of FXI and FXII activities of 35–40%. The *in vivo* effects on aPTT, FXI and FXII and Ir-CPI exposure level were consistent with the data obtained *in vitro* using rabbit plasma spiked with Ir-CPI.

Conclusion: These results demonstrate the antithrombotic potential of Ir-CPI in a model of AV-shunt in the rabbit whilst establishing the relationships between the antithrombotic efficacy, the Ir-CPI circulating concentration, the aPTT prolongation and the inhibition of FXI and FXII.

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PO182-TUE

surface plasmon resonance: SensiQ Pioneer as a means to screen drug discovery fragments for HITS

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Background: Common approaches to the identification of hits from small molecules or fragments are via high-throughput screening (HTS) assays and/or surface plasmon resonance (SPR).

Aims: The aims of this study were to validate hit identification between a HTS enzymatic assay and a high-throughput SPR system (SensiQ Pioneer).

Methods: A commercially-available fragment collection (1000 fragments) was employed to determine hits using the HTS enzymatic assay and the SensiQ Pioneer for binding against a target enzyme. The SensiQ Pioneer employs a single concentration of fragment and using a OneStep[®] programme allows for serial dilution of the fragment (analyte) within the association phase of the fragment to the target (ligand) to determine the affinity an interaction.

Results: When the data were compared between the two approaches, the fragment hits proved to be similar, with the majority of the hits from the enzyme assay being picked up by the SPR method and a few more binders to the target being identified from the SPR methodology.

Conclusion: The SPR technique using the SensiQ Pioneer is a useful tool for screening large libraries of small molecules/fragments to efficiently narrow down the number of hits for further analysis. The advantage of this SPR approach over other instruments is the ability to characterise affinity analysis within the first assay in addition to the identification of initial binders to the target. Furthermore, this instrument has the unique ability to perform OneStep[®] titration of the analyte eliminating the need to prepare a manual serial dilution of the analyte, meaning a single vial of one concentration can be employed. The software for the instrument is very easy to use allowing fast analyses of data. We conclude that SPR using the SensiQ Pioneer instru-

ment is an efficient and cost-effective method for fragment/compound screening.

Disclosure of Interest: None declared.

PO183-TUE

Modelling of surface-dependent activation of contact activation factor XII in a buffer solution

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Background: The process of factor XII (fXII) activation upon contact with a surface remains poorly understood, and there are several questions. Does fXII undergo spontaneous activation upon contact with a surface, or is it completely autoactivated by trace amounts of fXIIa? Does fXII bind to the surface to become autoactivated by fXIIa (bound-substrate model), or not (free-substrate model)?

Aims: We aimed to suggest putative mechanism of activation and to test spontaneous activation hypothesis, which refers to when binding with the surface alone causes formation of fXIIa.

Methods: We simulated experiments on the fXII activation in a buffer solution. We used kinetics law to derive a set of ODEs to be solved. With Copasi[®] software we calculated the system's time evolution under various conditions.

Results: We performed optimization procedures and considered mechanisms of autoactivation separately. Both mechanisms were able to qualitatively describe autoactivation. We obtained theoretical curves and overlaid the curves with dots corresponding to the experimental data, and "bound-substrate model" gave us better fitting.

To determine whether the system has the initial trace of an enzyme due to contamination of purified fXII used in activation studies or the system producing a sufficient amount of fXIIa via spontaneous activation we examined the total amount of fXIIa generated while varying surface concentration: if spontaneous activation occurs, activation goes to completion for a much narrower range of surface concentrations but its yield is larger upon excessive surface concentrations, and the fXII activation rate at large surface concentrations is non-zero, while it is zero without spontaneous activation.

Conclusion: Only the "bound-substrate model" describes the majority of experimental data, whereas the "free-substrate model" does not. As for the question of origin of initial fXIIa, measurable differences between the models with and without spontaneous activation appears only upon deficit or excess of the surface.

Disclosure of Interest: None declared.

PO184-TUE

Effect of a plant inhibitor of the contact system on a mouse thrombosis model

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Background: Many protease inhibitors in plants seeds interact with different classes of enzymes and contribute to modulation of biochemical pathways involved in inflammation, cancer, and thrombosis, among other pathological conditions. Trypsin, chymotrypsin, factor XIIa and plasma kallikrein are inhibited by *Enterolobium contortisiliquum* trypsin inhibitor (EcTI) that also prolongs partial activated thromboplastin time (aPTT).

Aims: To investigate the effect of the coagulation contact system inhibitor EcTI in a mouse thrombosis model.

Methods: The effect of EcTI was investigated in the photochemically induced thrombosis model in mice by monitoring the time course of the occlusion blood flow in an isolated carotid artery. We also investigated the inflammatory status induced by LPS prior to EcTI administration (2.5 mg kg⁻¹).

Results: In the groups treated with 2 or 4 mg kg⁻¹ EcTI prior to thrombus induction, the time of total artery occlusion was prolonged 58% and 96%, respectively, in relation to that of the control group (0.15 M NaCl). In the mesenteric microcirculation of the Wistar rats, EcTI reduced significantly cell behavior exacerbated in LPS-induced inflammatory response, which may indicate an involvement of this compound in the inhibition of the expression of adhesion molecules. In addition, the level of pro-inflammatory cytokines (TNF- α , MIP-1 α , IL-1 β , MCP-1) decreased significantly, demonstrating its anti-inflammatory action.

Conclusion: These data show that EcTI was able to prevent arterial thrombosis in mice. Further studies will be performed in order to clarify the mechanisms involved in EcTI action.

Disclosure of Interest: None declared.

Endothelium and vessel wall II

PO185-TUE

Endothelial cell increases its rigidity during inflammation through modification of the gap junction

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Background: Arterial stiffening along with aging, metabolic syndromes, and atherosclerosis represents a risk factor of cardiovascular diseases. Several studies have suggested a link between vessel stiffness and cardiovascular diseases. Vessel stiffness is determined by the rigidity of the multiple components in the extracellular matrix proteins, proteins and cells. Endothelial cellular stiffness has been shown to be affected by the substrate stiffness, shear stress, and other factors; however, the impact of vascular inflammation on endothelial cell stiffness remains poorly understood.

Aims: In this study, we investigated the regulation of endothelial cellular stiffness in the response to inflammatory stimulation.

Methods: To evaluate endothelial cell stiffness, we have measured the force curve of live human umbilical vein endothelial cells (HUVECs) by using atomic force microscopy.

Results: We have shown that stimulation of HUVECs with tumor necrosis factor- α (TNF- α) increased the endothelial cellular stiffness. The increase in stiffness was transient, thereby peaking at 4 h after stimulation and returning to the baseline stiffness after 24 h. Next we have observed that upon the formation of a confluent monolayer, HUVEC stiffness got decreased. This result led us to investigate how cell-cell interactions would contribute to endothelial cellular stiffness. Then we have shown that a gap junction inhibitor facilitates endothelial cell stiffening upon confluent monolayer formation.

Conclusion: Supported by our previous report that endothelial gap junction is suppressed after inflammatory stimulation, the present results suggest that endothelial cell increases the cellular stiffness in response to inflammatory stimuli by modifying the gap junctional intercellular communication. Our study provides a new insight into

the potential pathogenic role of endothelial cell stiffness driven by vascular inflammation.

Disclosure of Interest: None declared.

PO186-TUE

Analysis of the expression and localization of RNase and RNase inhibitor in blood cells and vascular endothelial cells involved in homeostasis in the vascular system

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Background: Extracellular RNA may be released from cells in cases of injury and vascular disease. It has been recognized as a novel procoagulatory and permeability-increasing factor and is counteracted by RNase 1 in endothelial cells.

Aims: No detailed characterization of the expression and localization of RNase and its inhibitor (RI) in the vasculature has been carried out so far. We aimed to investigate the expression and localization of RNase and RI in cells which come in contact with blood, such as platelets, mononuclear cells (MNCs), polymorphonuclear cells (PNCs), and red blood cells.

Methods: We investigated the expression and/or activity of RNase 1 and RI using western blot, RT-PCR, immunocytochemical staining, transmission electron microscopy, and RNase activity tests in blood cells, comparing them with those of the human umbilical vein endothelial cell line EAhy926. We further investigated the effect of anti-neoplastic drugs such as a proteasome inhibitor bortezomib on the expression of RNase 1 in EAhy926 cells.

Results: RNase 1 and RI mRNA and protein were similarly expressed in EAhy926 cells, MNCs and platelets, but RNase activity in supernatants derived from EAhy926 cells was much higher than in supernatants from any of blood cells. RNase 1 and von Willebrand factor (VWF) were partly colocalized in EAhy926. RNase may be protected from inhibition by RI in Weibel-Palade bodies and be released in the vasculature. While platelets release RNase 1 and RI with activation, RNase 1 was only partly colocalized with VWF in α -granules. RNase activity in supernatants derived from EAhy926 cells with or without dexamethasone and lenalidomide increased progressively, but it decreased gradually in supernatants from EAhy926 cells with bortezomib.

Conclusion: RNase activity may be mainly released by vascular endothelial cells in the vasculature, and RNase 1 released from blood cells seems to be inhibited by RI. The RNA/RNase 1/RI in blood cells may be a contributor to the regulation and maintenance of vascular homeostasis.

Disclosure of Interest: None declared.

PO187-TUE

Changes in the endothelial expression of genes related to inflammation and atherothrombosis in response to uremia

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Background: Endothelial dysfunction (ED) in chronic kidney disease (CKD) has a multifactorial etiology, with concomitant development of inflammation and oxidative stress.

Aims: In order to understand the molecular mechanisms involved in endothelial dysfunction in uremia, the effect of uremic media on the gene expression in endothelial cells in culture (ECs) has been investigated.

Methods: ECs were exposed to sera samples from patients on hemodialysis (HD) or healthy donors (C). Changes in gene expression were assessed using PCR profiler panels of genes related to atherosclerosis and inflammation. The differentially expressed genes were confirmed by RT-PCR using primers specifically designed, and their protein expression was verified by Western-blot.

Results: We observed changes in the expression of genes related to atherosclerosis (KLF2), proliferation (PDGFA, PDGFB), angiogenesis (PPARd) and inflammation (CCL2, VCAM1 and NFκB). By RT-PCR, we observed a significantly increased gene expression of VCAM1, CCL2 and PDGFB (fold increase of 3.8 ± 0.2 , 2.3 ± 0.1 , 2.1 ± 0.1 vs. C; $P < 0.05$), a moderate increase in NFκB and PDGFA (fold increase of 1.4 ± 0.3 and 1.3 ± 0.3 vs. C) and a reduced presence of KLF2 (fold increase 0.8 ± 0.2 vs. C). Protein expression followed the same patterns.

Conclusion: Exposure of endothelium to the uremic environment results in abnormal expression of genes related to inflammation and atherosclerosis, shifting to a proinflammatory phenotype. The decreased expression of the atheroprotective KLF2, together with the increased expression of proinflammatory genes, such as VCAM1 and NFκB, correlated with the proinflammatory phenotype observed in cells exposed to the uremic media. These changes could be involved in the development of the cardiovascular complications commonly associated with CKD.

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Disclosure of Interest: None declared.

PO188-TUE

Upregulation of matrix metalloproteinase-2 expression by activated protein C in human umbilical vein endothelial cells requires endothelial protein C receptor and activation of protease-activated receptors-1 and -3

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Background: Activated protein C (aPC), an enzyme in the coagulation cascade with important anticoagulant activities, has also been found to inhibit inflammation, promote angiogenesis, and enhance wound healing. APC can accelerate tissue matrix remodelling by directly stimulating matrix metalloproteinase (MMP)-2 activity and promoting the proliferation of human endothelial cells and keratinocytes.

Aims: The aim of the current study is to further elucidate the mechanism by which aPC regulates MMP-2.

Methods: MMP-2 gene and protein expression in cultured human umbilical-vein endothelial cells (HUVECs) was determined using reverse-transcriptional PCR, zymography and western blot, following the addition of relevant test agents.

Results: aPC, but not its inactive precursor protein C, dose-dependently upregulated MMP-2 gene and protein expression in HUVECs. Preventing the binding of aPC with its major receptor, endothelial protein C receptor (EPCR), by a blocking antibody, abolished its ability to upregulate MMP-2. Blocking antibodies to protease-activated receptors (PAR)-1 and to a lesser extent, PAR-3, also inhibited aPC-induced MMP-2 upregulation, whereas PAR-2 blocking antibody showed no effect. In concordance, activating peptides of PAR-1 and PAR-3, but not PAR-2, increased the expression of MMP-2.

Conclusion: Our results indicate that the interactions of aPC with EPCR, PAR-1 and PAR-3, but not PAR-2, are important for the

expression of MMP-2 on endothelial cells, which has implications for developing new therapeutic strategies to accelerate the tissue repair process during wound healing.

Disclosure of Interest: None declared.

PO189-TUE

Biomarkers of endothelial damage and inflammatory process in patients with Alzheimer disease or with mild cognitive impairment

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Background: Blood vessel damage and inflammation are important factors for Alzheimer disease (AD). Previous studies have reported higher levels of both soluble Intercellular Adhesion Molecule-1 (sICAM-1) and Tissue Factor Pathway Inhibitor (TFPI) due to endothelial damage, while soluble receptors of Tumoral Necrosis Factor (sTNFR1 and sTNFR2) have been described as important biomarkers of TNF-dependent inflammatory process.

Aims: To measure plasma levels of sICAM-1, TFPI, sTNFR1 and sTNFR2 in elderly patients with AD, mild cognitive impairment (MCI) and in elderly individuals without cognitive impairment (controls).

Methods: We evaluated 65 patients with AD, 55 with MCI and 30 controls. Plasma levels of sICAM-1, sTNFR1 and sTNFR2 were determined by ELISA using commercial kits, while TFPI was determined by an in-house ELISA. The results were expressed as median and interquartile range and analyzed by Kruskal–Wallis and Mann–Whitney tests.

Results: Levels of sICAM-1 were increased in AD compared to MCI and controls: 360.0 (174.0) vs. 298.0 (154.7) ng mL⁻¹, $P = 0.0091$; and 360.0 (174.0) vs. 273.4 (109.3) ng mL⁻¹, $P = 0.0005$, respectively. Levels of TFPI were elevated in AD compared to controls: 91.0 (39.0) vs. 69.0 (23.5)%, $P = 0.0025$, while sTNFR1 were increased in patients with both AD and MCI compared to controls: 2020 (1051) vs. 1313 (531) pg mL⁻¹, $P < 0.0001$; and 2020 (1051) vs. 1139 (506) pg mL⁻¹, $P < 0.0001$, respectively. Also sTNFR2 were increased in both AD and MCI compared to controls: 8015 (7026) vs. 2901 (1877) pg mL⁻¹, $P < 0.0001$; and 6124 (4544) vs. 2901 (1877) pg mL⁻¹, $P < 0.0001$, respectively. Significant correlation between sICAM-1 and sTNFR1 ($P = 0.029$, $r = 0.341$) and sTNFR2 ($P = 0.004$, $r = 0.435$) was observed only for DA patients.

Conclusion: Our data have confirmed that endothelial damage and proinflammatory factors are more evident in DA compared to MCI and controls while suggesting an association between inflammation and endothelial injury in the pathogenesis of AD. **Acknowledgment:** CNPq and FAPEMIG-Brazil.

Disclosure of Interest: None declared.

PO190-TUE

Activation of the endothelium may be involved in the hemostatic alterations induced by micrurus tener tener venom in C57BL/6 mice

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Background: We have previously described hemostatic activity induced *in vitro* by *Micrurus* venoms (Salazar et al 2011). Continuing these studies, we showed some hemostatic effects of *M. tener tener* venom (Mtt), including hemorrhagic manifestations and exacerbated endogenous fibrinolysis activation in a mouse model.

Aims: As endothelial activation may induce the disturbances seen in the *in vivo* model and due to the poor understanding of the hemostatic effects produced by these venoms, we evaluated the alterations in the vascular system.

Methods: C57BL/6 male mice paws were injected with 8 µg of Mtt. We confirmed the presence of alterations in the vascular system with a paw edema assay (Yamakawa et al 1976). Serum blood samples were collected at different times to evaluate cytokine release (TNF-α and IL-6). *Bothrops isabellae* venom (Bi) was used as a positive control and sterile saline solution as negative control. Then, different doses of Mtt were used to activate a mouse liver endothelial cell line (LSEC) after preliminary tests of the LD₅₀ (Vichai & Kirtikara, 2006). At 24 and 48 h, several parameters including expression or release of tissue factor (TF), von Willebrand factor (vWF), tissue type plasminogen activator (t-PA) and nitric oxide (NO) were evaluated, using thrombin and Bi as the positive controls.

Results: Mtt induced a moderate edematogenic effect at 240 min comparing with Bi at 120 min. The maximum levels of TNF-α and IL-6 were observed at 30 and 240 min, respectively. Mtt induced vWF and TF release *in vitro* with a pattern similar to Bi and thrombin, but did not induce t-PA and NO release as did both Bi and thrombin.

Conclusion: Mtt may produce hemorrhagic manifestations in the mice model through alterations in the vascular endothelium, which may be associated with the release of pro-inflammatory cytokines in addition to direct activation by the venom itself.

Disclosure of Interest: None declared.

PO191-TUE

Increased endocan levels and its association with severity in Stevens Johnson syndrome and toxic epidermal necrolysis

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Background: Stevens–Johnson syndrome (SJS) is a form of toxic epidermal necrolysis (TEN) in which cell death causes the epidermis to detach from the dermis. The pathophysiology of this syndrome is complex and involves endothelial dysfunction.

Aims: To determine the levels of endocan and inflammatory biomarkers in the systemic circulation of: 1) biopsy confirmed SJS/TEN subjects; 2) patients with allergic skin reactions but biopsy negative for SJS/TEN; and 3) normals.

Methods: Sixteen patients > 18 years were recruited and 7 confirmed for SJS/TEN. Plasmas were assayed for endocan, tumor necrosis factor-α (TNFα), vascular endothelial growth factor (VEGF), and C-reactive protein (CRP). Differences between SJS/TEN, biopsy negative (B–), and healthy (H) (*n* = 23) subjects, explored using ANOVA and Tukey's *post-hoc* test. Data presented as median [IQR].

Results: SJS/TEN and B– subjects had higher endocan levels than normal controls (SJS/TEN: 3.0 ng mL^{−1} [2.2–8.1]; B–: 4.0 ng mL^{−1} [1.5–4.9]; normal controls: 1.8 ng mL^{−1} [1.7–2.0]; *P* = 0.004). Endocan levels were more strongly associated with SCORTEN in SJS/TEN subjects than in B– subjects (*r*-squared SJS/TEN = 0.51; B– = 0.03). SJS/TEN subjects exhibited significantly higher levels of TNFα compared to H subjects (*P* = 0.03). SJS/TEN TNFα levels were significantly lower compared to B– subjects (*P* = 0.005). VEGF levels were also elevated among SJS/TEN and B– subjects compared to H subjects (SJS/TEN: 12.0 pg mL^{−1} [7.6–52.7]; B–: 10.5 pg mL^{−1} [8.7–12.0]; H: 4.9 pg mL^{−1} [4.2–6.5]; *P* < 0.0001). There was no significant difference in VEGF levels between SJS/TEN and B– subjects. Similarly, CRP levels were elevated among SJS/TEN and B– compared to H subjects. CRP levels between SJS/TEN and B– did not differ significantly.

Conclusion: This is the first study to evaluate endocan in systemic circulation of SJS/TEN. Elevated endocan levels were more strongly associated with severity among SJS/TEN than among less severe allergic reactions with skin involvement.

Disclosure of Interest: None declared.

PO192-TUE

Endothelial pathogenesis results in the generation of a proteoglycan Endocan in various diseases

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Background: Endocan is a proteoglycan which is upregulated in various diseases involving endothelial dysfunction. Pro-inflammatory mediators such as TNFα, IL1β and bacterial lipopolysaccharide (LPS) and VEGF and FGF-2 promotes its generation. Endocan binds to LFA-1 and inhibit transendothelial migration of leucocytes during inflammation.

Aims: The aim of this study is to quantitate endocan levels in various diseases in which endothelial function is dysregulated.

Methods: Citrated plasma samples were obtained from 30 disseminated intravascular coagulopathy (DIC), 68 end stage renal disease (ESRD), 7 stevens johnson syndrome/toxic epidermal necrolysis (SJS/TEN), and 51 liver disease patients. Endocan/ESM-1 was quantitatively measured using an enzyme-linked immunoassay (ELISA) according to the manufacturer's protocol (LUNGINNOV s.a.s. Lille, France). The differences in endocan levels between DIC, ESRD, SJS/TEN, liver disease, and healthy normals (*n* = 42) were assessed using kruskal–wallis ANOVA and Dunn's *post-hoc* test. Data are presented as median [IQR].

Results: Endocan levels were significantly different between DIC (3.3 ng mL^{−1} [2.3–6.4]), ESRD (2.2 ng mL^{−1} [1.6–3.0]), SJS/TEN (3.0 ng mL^{−1} [2.1–8.1]), liver disease (4.3 ng mL^{−1} [1.4–9.3]), and healthy normals (1.8 ng mL^{−1} [1.6–2.0]) (ANOVA *P* < 0.0001). Dunn's *post-hoc* test comparing each of the diseased groups to healthy normals revealed that endocan levels were significantly increased among DIC (*P* < 0.0001), ESRD (*P* = 0.005), and SJS/TEN (*P* = 0.005). No significant difference was observed between liver disease and healthy normals (*P* = 0.6023).

Conclusion: These studies demonstrate that endocan is increased in diseases in which endothelial function is altered due to various pathogenic mechanisms. Such diseases as DIC, SJS/TEN and ESRD showed marked elevation in endocan levels in comparison to the normal. This data suggest that endocan can be used as a prognostic marker of endothelial dysfunction.

Disclosure of Interest: None declared.

PO193-TUE

Upregulation of chemokine expression by *Streptococcus mutans* bearing collagen-binding protein in cultured brain endothelial cells

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Background: We previously reported that infection with *Streptococcus mutans* (*S. mutans*) expressing collagen-binding protein (CBP) further aggravates cerebral haemorrhage in a mouse stroke model, which may result from the activation of matrix metalloprotease-9 (MMP-9).

Aims: In the present study, we investigated whether the CBP of *S. mutans* contributes to the expression of MMP-9 and chemokines using cultured brain endothelial cells.

Methods: A transformed mouse brain endothelial cell line, b.End3, was exposed to serotype *k* *S. mutans* (TW295) or TW295CND (a CBP-deficient strain generated by inactivation of gene encoding CBP in TW295), or recombinant Cnm protein (CBP). After the exposure, the mRNA expression of MMP-9 and chemokines CCL2 and functional IL-8 homologues CXCL1/KC and CXCL2/MIP-2 was assessed by real-time PCR.

Results: The expression of MMP-9 mRNA was not changed in both TW295 and TW295CND groups. In contrast, the mRNA expression of CCL2, CXCL1 and CXCL2 was significantly enhanced by the exposure to TW295 but not TW295CND. Recombinant Cnm protein (CBP) itself also upregulated the mRNA expression of CCL2, CXCL1 and CXCL2.

Conclusion: Thus, the CBP of *S. mutans* TW295 is important for the augmentation of expression of chemokines (CCL2, CXCL1, CXCL2) that may secondarily result in MMP-9 activation.

Disclosure of Interest: Y. Matsumoto: None declared, K. Hokamura: None declared, K. Umemura Grant/Research Support from: JSPS A Grant-in-Aid for Scientific Research (B) No. 24390138.

Fibrinogen / fibrin / D-dimer II

PO194-TUE

Two novel different substitutions of fibrinogen Aalpha 98Kotlín R¹, Reicheltova-Riedelova Z¹, Ceznerová E¹, Štikarová J¹, Pastva O¹, Suttar J¹, Salaj P² and Dyr JE¹¹Department of Biochemistry; ²Center for Thrombosis and Hemostasis, Institute of Hematology and Blood Transfusion, Prague 2, Czech Republic

Background: Hereditary dysfibrinogenemia is a disease wherein an inherited abnormality in fibrinogen molecule results in defective fibrin function.

Aims: The aim of our study was to characterize a cause of an abnormal coagulation in two unrelated patients with mutation in fibrinogen Aα 98.

Methods: Fibrin polymerization and fibrinolysis were measured by turbidimetric method. Kinetics of fibrinopeptide release was measured by HPLC. Gene sequencing was performed by dideoxysequencing method.

Results: The first patient was a 27-year old woman with decreased functional fibrinogen level and history of thrombosis. Total fibrinogen level, thrombin and reptilase times were within normal range. Genetic analysis revealed a heterozygous substitution Aα Phe98Tyr. The second patient was a 42-year old woman with decreased functional fibrinogen level, two consecutive abortions, no bleeding symptoms apart from mild menorrhagia, and no history of thrombosis. Genetic analysis revealed a heterozygous substitution Aα Phe98Ile. Both patients

presented with normal kinetics of fibrinopeptide release. Fibrin polymerization and fibrinolysis were impaired in both cases.

Conclusion: Both patients were found to be heterozygous for a point mutation in fibrinogen Aalpha 98. Mutations are situated in the coiled-coil connector. The amino acid substitutions are located in the middle of the coiled-coil connector of fibrinogen in the RGDF sequence Aα 95–98. Mutations are situated in close proximity to the plasmin cleavage site and the cryptic site, which affects the lateral association of protofibrils. Although both mutations are situated at the same site they had a different clinical manifestation. This work was supported by the project of the Ministry of Health, Czech Republic, for conceptual development of research organization 00023736, by Grant from the Grant Agency of the Czech Republic nr. P205/12/G118, and by ERDF OPK CZ.2.16/3.1.00/24001.

Disclosure of Interest: None declared.

PO195-TUE

Clinical features and molecular basis of 102 Chinese patients with congenital dysfibrinogenemiaZhou J¹, Ding Q², Chen Y³, Ouyang Q¹, Jiang L¹, Dai J², Lu Y², Wu X², Liang Q², Wang H¹ and Wang X²¹Shanghai Institute of Hematology, Ruijin Hospital Affiliated To Shanghai Jiaotong University School of Medicine Shanghai, China; ²Department of Laboratory Medicine, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai; ³Department of Laboratory Medicine, The 303 hospital of Chinese People's Liberation Army, Nanning, China

Background: Congenital dysfibrinogenemia (CD) is a rare qualitative disorder of fibrinogen (Fg) with heterogeneous clinical manifestations.

Aims: We aimed to analyze clinical phenotype and molecular basis of 102 Chinese patients with CD and to evaluate the application of thrombolastography (TEG) in CD patients.

Methods: Clinical manifestations were recorded and quantified using the consensus ISTH bleeding assessment tool. Kaolin activated TEG and functional Fg TEG (FF-TEG) were applied in 30 CD patients and 20 normal controls. Genetic analysis of the three fibrinogen genes (*FGA*, *FGB*, *FGG*) were performed by direct sequencing.

Results: 27.5% CD patients (female 19/28) experienced bleeding, 3.9% had thrombosis and 68.6% were asymptomatic. Significant difference ($P < 0.05$) in parameters of both TEG were found between patients with mutations at AαArg35(16) and γArg301(275), but were not identified between patients with and without bleeding. TEG results were normal in CD patients with mutations at Arg35(16), Pro37(18) or Arg38(19) on Aα chain. A total of 18 mutations were identified and 6 of them are novel, including Gly33(14)del, Asp57(38)_Trp60(41)del IVS2+1_+2GTdel and Phe742(723)Tyr on Aα chain, and Asn334(308)Thr, Gly335(309)Cys and Trp395(369)Leu on γ chain.

Conclusion: Most CD patients were asymptomatic and female were more prone to experience bleeding than male. Parameters in TEG could not indicate the bleeding risk in CD patients, but priority of mutation screening at thrombin cleavage site or polymerization site on Aα chain may be given if TEG results are normal in CD patients.

Disclosure of Interest: None declared.

PO196-TUE

Coexistence of congenital fibrinogen deficiency and hereditary protein C deficiency in an infant presenting with purpura fulminans

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Background: Protein C is the key component of the anticoagulant system. Hereditary protein C deficiency causes a hypercoagulable state. Clinical presentation of protein C deficiency varies from severe neonatal purpura fulminans, or life-threatening thrombotic disorder to asymptomatic. The incidence of heterozygous protein C deficiency is 1/200–500 healthy individuals, whereas homozygous protein C deficiency incidence is estimated at 1/500,000–750,000. Congenital fibrinogen deficiency is an extremely rare hereditary bleeding disorder, affecting 1 in 1,000,000 individuals.

Aims: Combination of both has been reported in very few cases.

Methods: A male infant aged 9 months was presented with the complaint of bruising on his legs. The physical examination revealed ecchymosis with consolidated irregular areas of blue black hemorrhagic necrosis on his lower limbs and lower gluteal area. Purpura fulminans was considered based on the cutaneous findings.

Results: Laboratory examinations revealed anemia and thrombocytopenia. PT and PTT were prolonged, fibrinogen was low and D-dimer was high. These findings supported disseminated intravascular coagulation. Protein C activity was low. He was treated with heparin and fresh frozen plasma which helped him to recover clinically. He was discharged with still low protein C activity and low fibrinogen level. These levels did not return to normal by the next follow-up visit. Protein C levels ranged from 10 to 20%. Since he had fibrinogen levels $<50 \text{ mg dL}^{-1}$ and recurrent soft tissue bleeding, prophylaxis was conducted with fibrinogen concentrate $30 \text{ mg kg}^{-1}/\text{dose}$ weekly. Our patient had no more thromboembolic event or bleeding symptoms during the treatment with TDP and fibrinogen concentrate.

Conclusion: Based on these findings, Protein C deficiency and fibrinogen deficiency were diagnosed and a blood sample was sent for genetic diagnosis.

Disclosure of Interest: None declared.

PO197-TUE

International study to assess the performances of a new liquid assay for determination of fibrinogen

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Background: The Clauss method for fibrinogen testing appears to be the most reliable method for general use in clinical laboratories. A new assay reagent for the quantitative determination of fibrinogen levels in plasma, based on the Clauss clotting method, was developed in a liquid format to improve its convenience.

Aims: Evaluate the performances of the new reagent through a method comparison on results obtained in 3 different laboratories: Gainesville (USA), Marseille (France) and Cremona (Italy).

Methods: Patients were tested with the new reagent: STA[®] – Liquid Fib (Diagnostics Stago, Asnières s/Seine, France) (LiqFib) and the reference method: STA[®] – Fibrinogen 5 (Diagnostics Stago) (Fib5) on STA-R[®] or STA[®] Compact analyzers (Diagnostics Stago) using pre-calibrated test setups. Four hundred and thirteen patients referred to the laboratory for fibrinogen testing were included in the study.

Seventy-eight samples had fibrinogen levels lower than 1.5 g L^{-1} and 66 samples had fibrinogen levels above 8 g L^{-1} . Samples were spread along the working range ($0.4\text{--}12 \text{ g L}^{-1}$). Correlations were analyzed through linear regressions (LiqFib versus Fib5) and Bland & Altman Graph.

Results: Results obtained with the new reagent LiqFib versus the current reagent Fib5, showed an excellent correlation in the three laboratories:

Gainesville: $y = 0.94x + 0.14$ ($r = 0.998$).

Marseille: $y = 0.96x + 0.22$ ($r = 0.994$).

Cremona: $y = 1.01x + 0.10$ ($r = 0.997$).

When data from the three sites were combined, the correlation was still optimal ($r = 0.995$).

Bland & Altman analysis showed more than 95% of samples within our analytical acceptable ranges.

Conclusion: The comparison between the new reagent, LiqFib, and the reference reagent, Fib5, demonstrates the excellent correlation of results for the whole assay working range. In addition, as LiqFib is a ready to use liquid reagent, it can improve workability in the laboratory.

Disclosure of Interest: N. Harris Grant/Research Support from: Stago, J. Valadier Grant/Research Support from: Stago, S. Testa Grant/Research Support from: Stago.

PO198-TUE

Pharmacokinetic (PK) comparison of two fibrinogen concentrates for the treatment of congenital fibrinogen deficiency

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Background: Patients with congenital afibrinogenemia and hypofibrinogenemia, can experience severe bleeding episodes starting at birth or early childhood. Therapeutic substitution with human fibrinogen concentrate can correct the haemostatic defect and arrest the bleeding in patients with these fibrinogen deficiencies.

Aims: A new plasma derived, highly purified, lyophilized, fibrinogen concentrate, that includes two dedicated virus inactivation/removal steps is compared to another commercially available product.

Methods: This ongoing study is a prospective, randomized, open-label, multinational, crossover PK comparison of two fibrinogen concentrates, including comparison of a surrogate efficacy endpoint Maximum Clot Firmness (MCF) measured by ROTEM[®]. The study includes a crossover design where both products are given a single dose in a randomized fashion separated by an observation period and wash-out of any fibrinogen product prior to infusion.

Results: Nine adult and adolescent patients were included in a planned interim analysis. Comparable PK profiles between the products were seen in the interim analysis but in key parameters, Normalized Area Under the Curve (AUC_{norm}), the new concentrate 0.994, marketed concentrate $0.731 \text{ h-mg mL}^{-1}/(\text{mg kg}^{-1})$, P -value 0.014) and clearance (new 0.522, marketed $0.594 \text{ mL h}^{-1} \text{ kg}^{-1}$, P -value 0.033), significant differences were observed between the groups. Comparable haemostatic efficacy of the two products was demonstrated based on their ability to significantly increase MCF.

Conclusion: The interim data from this study showed in general a comparable PK profile for the two concentrates in patients with congenital fibrinogen deficiency. The new concentrate showed a significantly higher AUC_{norm} and lower clearance than the comparator. The haemostatic efficacy of the new concentrate, as measured by change in MCF as a surrogate parameter, was similar to that of the licensed

comparator, and there was no related AE or SAE for the new concentrate after single-dose administration.

Disclosure of Interest: B. Schwartz Employee of: Octapharma, S. Rangarajan: None declared, M. Karimi: None declared, S. Knaub Employee of: Octapharma, F. Peyvandi: None declared.

PO199-TUE

Fibrinogen and factor XIII are modified by N-homocysteinylation reactions

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Background: Homocysteine thiolactone (HTL), one of the compounds responsible for the harmful effects associated to hyperhomocysteinemia, can react with biological compounds (N-homocysteinylation). In previous studies we demonstrated that this process alters the kinetics of plasma fibrin formation and lysis and modifies the structure of the networks.

Aims: To study effects of HTL on fibrinogen (Fbg) and factor XIII (FXIII).

Methods: Fbg and FXIII were incubated with HTL (molar ratio protein:HTL = 1:500 and 1:1000; 3 h, 37 °C) or saline buffer as control. *Capillary Zone Electrophoresis (CZE):* N-homocysteinylation products (Fbg_{HTL} and FXIII_{HTL}) were analyzed and electroferograms were registered. *Fibrinof ormation:* clots were generated by adding thrombin and CaCl₂ to the Fbg_{HTL}. Optical densities (OD) at 405 nm were measured. The sigmoid curves were characterized by lag phase, maximum velocity (V_{max}) and final network OD. *Clot lysis:* fibrin networks were obtained in the presence of plasminogen and tislular plasminogen activator. Fifty percentage lysis times were recorded. *FXIII activity (photometric method):* decreases of OD (Δ_{OD}) vs. time were registered. All assays were performed in quadruplicate.

Results: CZE: electroferograms of Fbg_{HTL} and FXIII_{HTL} showed enhanced migration times vs. control. *Fibrinof ormation assays:* Fbg: HTL (1:1000) showed lag phase 5.8 ± 0.1 min vs. 2.63 ± 0.02 min; V_{max} 19.75 ± 1.48 min⁻¹ vs. 59.25 ± 2.77 min⁻¹ and final network OD 0.865 ± 0.008 vs. 0.920 ± 0.021 . *Lysis 50%:* 22.00 ± 0.40 min vs. 15.33 ± 0.46 min. *FXIII activity:* Δ_{OD} were 0.0487 ± 0.0037 vs. 0.0389 ± 0.0014 . In all assays significant differences ($P < 0.01$) between treated samples and controls were observed.

Conclusion: N-homocysteinylation reactions induced structural changes in Fbg and FXIII molecules. Fbg_{HTL} impaired fibrinof ormation kinetics and prolonged lysis time respect to control. HTL increased FXIII transglutaminase activity. These results would explain, at least partially, the findings observed in plasma system.

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Disclosure of Interest: None declared.

PO200-TUE

A case of thrombosis in congenital afibrinogenemia patient: a physiopathologic and management dilemma

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Background: In Congenital Afibrinogenemia (CA) arterial and venous thromboembolism can occur spontaneously or following fibrinogen replacement therapy.

Aims: We describe the case of a CA male patient who experienced an arterial thrombosis in his left lower limb.

Methods: The patient, born in 1973, during his life presented mild moderate bleeding episodes treated first with cryoprecipitate, and since 1978 with fibrinogen concentrate (FC).

Results: In October 2008 after a trauma of lower left limb, he developed a thrombosis of left anterior and posterior tibial arteries. Treatment with low molecular weight heparin 200 IU kg⁻¹ day⁻¹, Iloprost and FC (as anti hemorrhagic prophylaxis) was administered. In December 2008, amputation of left foot toes was performed because of necrosis. Biopsy of pedial arterial vessels showed a concentric myointimal hyperplasia. In the next months, despite medical treatment, the arterial thrombosis progressed to the obstruction of the whole left arterial district (iliac-phenoral-popliteal-tibial arteries). At that point, anti hemorrhagic prophylaxis with FC was substituted with Fresh Frozen Plasma (FFP). As an extreme attempt to reduce the obstruction, an arteriography was performed to place a surgical bypass and to try local thrombolysis with urokinase. Although a transitory amelioration, at day 3 the US demonstrated the occlusion of the stent and of the whole arterial left district, progressed to the aorto-iliac bifurcation. In June 2009 the vascular surgeon amputated the left lower limb because of complete necrosis. Surgery was performed under FFP without bleeding complications. After surgery patient continued to be treated with enoxaparin 100 IU kg⁻¹ day⁻¹, clopidogrel 75 mg day⁻¹, aspirin 100 mg day⁻¹, FFP administered twice a week. Since then the patient has not experienced other thrombotic or hemorrhagic events.

Conclusion: This case demonstrates the complexity of physiopathology and clinical management of a 'so called' bleeding disorder as CA.

Disclosure of Interest: None declared.

PO201-TUE

CTEPH-associated dysfibrinogenemia with enhanced accessibility on fibrin of ligands for VE-cadherin

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Background: CTEPH-associated dysfibrinogenemias (fibrinogens_{San Diego I-V}) produce fibrin clots (FC) that are somewhat resistant to fibrinolysis. They also have altered molecular and polymeric structures, although it is unclear how the alterations might influence remodeling of thrombi into vascular scars. The fibrin β -chain amino terminus (B knob) binds the endothelial cell (EC) transmembrane receptor, VE-cadherin, which has been implicated in vascular remodeling. Prolonged or enhanced B knob accessibility in CTEPH-associated fibrin could augment EC signaling and influence vascular scarring in the presence of pulmonary emboli (PE).

Aims: To determine if FC derived from CTEPH-associated dysfibrinogenemias expose an increased number of B-knobs compared to normal fibrin.

Methods: FC were formed from 2 CTEPH-associated fibrinogens: fibrinogen_{San Diego-III} (in which plasmin access to the lytic target is limited) and fibrinogen_{San Diego-V} (in which a net negative charge imparted to fibrin might disrupt polymer formation). Negative controls were FC derived from healthy controls confirmed to have normal fibrinogen. Positive controls were FC formed under hypertonic conditions, which disrupts polymer formation. Anti-B knob and isotypic control antibodies (Ab) were labeled with ¹²⁵I and then percolated through FC. After washes, retained ¹²⁵I-labeled Ab were measured by gamma counting.

Results: FC from fibrinogen_{San Diego-V} and FC formed under hypertonic conditions retained more anti-B knob than the FC formed under

isotonic conditions from normal fibrinogen. FC from fibrinogen_{San Diego-III} retained anti-B knob similar to clots from normal fibrinogen. None of the clots retained the isotype control Ab.

Conclusion: FC formed from fibrinogen_{San Diego-V} had more accessible B knobs: promoting VE-cadherin mediated EC activation may explain vascular scars after PE in these patients. Fibrinogen_{San Diego-III} may have a different mechanism of clot persistence and scar formation.

Disclosure of Interest: None declared.

PO202-TUE

Non-enzymatic fibrinogen self-assembly unveiled

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Background: Fibrinogen (fg) self-assembly (FSA) can be induced by centrifugation, protamine sulfate precipitation, Factor XIIIa-cross-linking, and hydrophobic adsorption. Apart from fibrin-identical fiber periodicity, and intermolecular α C- α C contact dependence, its mechanism is unknown. Two minor fractions of fg isolates, one possessing FSA capacity and the other comprising soluble fibrin/fibrinogen complexes (FFC), led us to hypothesize that FFC play an FSA role.

Aims: To test this hypothesis.

Methods: Isolated fg, >97% clottable, was PMSF-treated to neutralize trace proteases. Fg (4 mg mL⁻¹, μ = 0.15, pH 7.4, 0.5 mM EDTA) was incubated overnight with polystyrene(PS)-coated 1 × 1 cm silica wafer plates. Resulting surface images were assessed by light microscopy and atomic force microscopy topographic scans. To induce FSA by flow, fg (4 mg mL⁻¹, pH 7.4, μ = 0.15) was subjected to peristalsis in a 17.5 ml capacity circular 3/16" ID Tygon tube, at 100 mL min⁻¹, 12 h. The interior tube surface consisted of PS or polylactic acid (respective water droplet angles 90° and 70°). To induce FSA by static procedure, fg was subjected to μ = 0.05, pH 6.4, 4° overnight.

Results: By either FSA inducing procedure, resulting clots represented 6–9% of total fg, n = 4. By SDS-PAGE all clots displayed intact chains with minor/trace amounts of $\gamma\gamma$ dimers. PS surface-linked images of pre-clot fg displayed numerous variable size aggregates of fiber networks. Those of clot supernatant fg were markedly diminished (to <10%), as were those of pre-clot fg pre-incubated with 5 molar excess fg fragment D100, or 100 molar excess GPRP peptide. These and pre-clot fg networks were morphologically similar, indicating incomplete FSA inhibition.

Conclusion: The results imply a critical FFC role in FSA. Also, they underscore that FFC most likely account for 'cryofibrinogenemia', and that they possibly coalesce/localize into insoluble clots at non-endothelial including implanted device and atheroma surfaces.

Disclosure of Interest: None declared.

PO203-TUE

Assessment of a test for functional fibrinogen in whole blood using ultrasound technology compared to claus fibrinogen in plasma

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Background: Currently no point-of-care (POC) device exists to measure fibrinogen levels in whole blood that correlate well with fibrinogen estimates based on the Clauss method. Assessment of fibrinogen is typically performed in the central lab using plasma, resulting in long turn

around times. This results in the sub-optimal selection of transfusion products for the management of bleeding.

Aims: To assess the feasibility of the QuantraDx™ System, a novel POC device that uses an ultrasound based technology, to determine fibrinogen concentrations in whole blood compared to a Clauss fibrinogen method.

Methods: Native plasma in whole blood from 5 healthy volunteers was replaced with Factor Assay Control plasma (FACT) with varying levels of fibrinogen. The levels, ranging from 286 to 75 mg dL⁻¹, were made by mixing FACT with a known fibrinogen level and fibrinogen deficient plasma. The samples were run on the QuantraDx where time to clot and clot stiffness were measured using ultrasound. The same samples were also evaluated on the STAGO Start4 using the STAGO Fibrin-Test kit. A saturating amount of Reopro® was used to suppress the platelets for all samples tested on the QuantraDx. Clotting was initiated with dilute tissue factor and the anticoagulant reversed with calcium chloride.

Results: QuantraDx results demonstrated a linear relationship between clot stiffness and theoretical fibrinogen levels ranging from 286 to 75 mg dL⁻¹. Good agreement was achieved when comparing the STAGO Clauss fibrinogen concentration to the shear modulus values from the QuantraDx (R values ranged from 0.924 to 0.989) across the sample population tested). Duplicate precision for the QuantraDx (6.5% CV, n = 27) was higher than for the Clauss Method (3.4% CV, n = 27) but still acceptable for use in the critical care setting.

Conclusion: This study demonstrates that it is feasible to develop a whole blood method for determining fibrinogen concentration based on the QuantraDX System that correlates with a traditional Clauss fibrinogen method.

Disclosure of Interest: K. Blasier Shareholder of: Hemosonics, Employee of: Hemosonics, E. Ferrante Shareholder of: Hemosonics, Employee of: Hemosonics, J. Terrasi Employee of: Hemosonics, T. Fischer Shareholder of: Hemosonics, Employee of: Hemosonics, F. Viola Shareholder of: Hemosonics, Employee of: Hemosonics.

Hemophilia clinical II

PO204-TUE

Haemophilia presentations...let me count the ways

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Background: Haemophilia is an X-linked disorder often diagnosed due to a known family history although spontaneous mutations do arise with diagnosis after easy bruising and/or bleeding episodes.

Aims: Here we describe 4 interesting presentations of haemophilia diagnosed over the last few years.

Methods: Nil.

Results: Case 1 with no known history of haemophilia was detected preoperatively due to a prolonged APTT and was subsequently shown to have a previously unrecognised mutation 1763 A>T resulting in moderate haemophilia. The mutation was also found in mother but not grandfather.

Case 2 was initially believed to be a non-accidental injury in a girl with a knee effusion. She was subsequently found to be a Turner syndrome carrying a 5123 A>G mutation conferring mild haemophilia.

Case 3 was unusual in that haemophilia was found in the son of a haemophilia patient who by chance had married a haemophilia carrier.

Case 4 was only diagnosed due to gene analysis as his factor VIII level was normal. He was the son of a known carrier and tested due to this history but was found to be a Klinefelter and analysis showed he is in fact a male carrier of haemophilia.

Conclusion: The possible form of presentation of haemophilia cannot be underestimated.

Disclosure of Interest: None declared.

PO205-TUE

Intracranial hemorrhage in early neonates with hemophilia in JapanTaki M¹, Shima M² and on behalf of J-HIS2 Study Group¹Department of Pediatrics, St. Marianna University School of Medicine Yokohama-city Seibu Hospital, Yokohama;²Department of Pediatrics, Nara Medical University, Kashihara, Japan

Background: Intracranial hemorrhage (ICH) at delivery in hemophilia patients continues to be a major problem, particularly when coupled with a lack of information as to whether or not mothers are hemophilia carriers.

Aims: In this study, we investigated the associations among family history of hemophilia, mode of delivery, and ICH in early neonates.

Methods: The subjects were a total of 245 patients registered in the prospective patient registration system for those newly diagnosed with hemophilia (J-HIS 2) which had started in 2008, including 213 patients with hemophilia A and 32 with hemophilia B. We retrospectively studied patients who suffered ICH within 1 week after birth in terms of the mode of delivery and family history of hemophilia.

Results: 1) In total, 173 patients were delivered vaginally (VD) and 59 by cesarean section (CS). Of the 173 patients delivered by VD, 147 were delivered spontaneously, 16 by vacuum extraction, and 1 by forceps, whereas the mode of delivery was unknown in nine patients. CS was chosen based on the possibility of hemophilia in the infant in seven cases, for obstetrical reasons in 38, and for unknown reasons in 14. ICH occurred within 1 week after birth in five patients delivered by VD (2.9%) and 1 by CS (1.7%). Of these five patients delivered by VD, 4 were delivered by vacuum extraction and 1 spontaneously. 2) of 129 patients without a family history, 105 were delivered by VD (81.4%) and 24 by CS (18.6%). Of 97 patients with a family history, 63 (64.9%) were delivered by VD and 34 (35.1%) by CS. Eight patients with a family history (7.6%) and 8 without a family history (12.7%) were delivered by vacuum extraction or using forceps.

Conclusion: The incidence of ICH did not differ distinctly between patients delivered spontaneously by VD and those delivered by CS. However, it was confirmed that the risk of ICH is high among patients delivered vaginally by vacuum extraction. The study suggested a higher tendency to select CS for mothers with a family history.

Disclosure of Interest: None declared.

PO206-TUE

A Phase III, open-label, multicenter study to evaluate pharmacokinetics of a plasma-derived von willebrand factor/factor VIII (VWF/FVIII) concentrate in pediatric subjects with haemophilia A (SWIFTLY-HA STUDY)Pompa MT¹, Iosava G², Khayat C³, Romashevskaya I⁴, Aleinikova O⁵, Stasyshyn O⁶, Vashchenko L⁷, Lopez MJ⁸ and Seifert W⁹¹Monterrey Nuevo Leon OCA Hospital (MIRC), MonterreyNuevo Leon, Mexico; ²Joint Stock Hematology andTransfusiology Research Institute, Tbilisi, Georgia; ³HospitalHôtel Dieu de France, Beirut, Lebanon; ⁴Republican Research

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Background: Voncento[®] comes with a low-volume and the solution contains a large proportion of high-molecular-weight VWF multimers

with a VWF:FVIII ratio of 2.4:1. The SWIFT ('Studies with von Willebrand factor/Factor VIII') program is evaluating this product in hemophilia A and VWD patients in accordance with the European clinical and pediatric guidelines.

Aims: This was a Phase III study to investigate the pharmacokinetics (PK) in pediatric subjects aged 0 to <12 years with severe hemophilia A (FVIII:C < 1%) who had received previous FVIII treatment for a minimum of 20 exposure days. The efficacy and safety data are presented in abstract *ISTH15ABS-1336*.

Methods: Subjects received Voncento[®] as a single bolus infusion of 50 IU FVIII kg⁻¹ on Day 1 (*n* = 33: 15 subjects were <6 years including 3 subjects <2 years). PK parameters for FVIII were derived from plasma concentration values collected prior to dosing and then at 0.5, 4, 8, 24, and 48 h after the infusion. PK parameters comprised Incremental recovery (IR), Half-life (*t*_{1/2}), Area under the concentration curve (AUC), Maximum plasma concentration (*C*_{max}), Minimum plasma concentration (*C*_{min}), Time to maximum concentration (*t*_{max}), Mean residence time (MRT), Clearance (CL), Volume of distribution at steady state (*V*_{ss}).

Results: In the overall PK population, a mean *C*_{max} of 0.76 IU mL⁻¹ was found at a median of 35.0 min post-dose, which then declined over time with mean terminal *t*_{1/2} of 10.7 h and MRT of 14.3 h. Mean IR was 0.015 (IU mL⁻¹)/(IU kg⁻¹), mean CL was 6.02 mL h⁻¹ kg⁻¹, and mean *V*_{ss} was 87.6 mL kg⁻¹. Mean values for PK parameters were slightly higher in the 6 to <12 years age group compared to the 0 to <6 years age group (with the exception of CL).

Conclusion: Concentration-time curves showed similar profiles for the subjects aged <6 years and those aged 6- < 12 years, but, as expected, the youngest of the children have an increased clearance. A contemporary comprehensive development program evaluating Voncento[®] across all ages in hemophilia A is now available.

Disclosure of Interest: M. T. Pompa: None declared, G. Iosava: None declared, C. Khayat: None declared, I. Romashevskaya: None declared, O. Aleinikova: None declared, O. Stasyshyn: None declared, L. Vashchenko: None declared, M. J. Lopez: None declared, W. Seifert Employee of: CSL Behring.

PO207-TUE

National Inhibitor Survey in Haemophilia Patients (NISH) from India

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Background: The development of alloantibodies to clotting factors is a serious complication in patients with bleeding disorders. The mechanism of inhibitor development is thought to be complex, but a timely diagnosis can be life-saving.

Aims: The aims of this study were to diagnose Indian patients with bleeding disorders, compare the inhibitor incidence in different regions, create a national database with the patient information, and analyse patient and treatment-related variables.

Methods: Citrated blood samples were collected at different centres in India after informed consent. Patient details were recorded, coagulation and inhibitor assays were performed.

Results: Out of the 4783 samples analysed, 4704 had bleeding manifestations. 276/3884 (7.11%) were 'FVIII Inhibitor positive' among the Haemophilia A (HA) patients, and 276/3371 (8.19%) among the severe HA patients, mostly 'high-responders'. Inhibitor incidence varied from 3.26% to 11.94% in different regions. Six FIX Inhibitor positive patients were diagnosed i.e. 0.98% of 611 Haemophilia B (HB) patients and 1.12% of 535 severe HB patients. Inhibitors were not detected in those with other rare bleeding disorders. Indian haemophiliacs are mostly treated 'on-demand', and haemarthroses continues to

be the most significant bleeding manifestation. Treatment products in the patients varied from either factor concentrates or blood products to a combination of both.

Conclusion: This study, the first national survey of its kind has resulted in the initiation of maintaining a database with regard to patients' inhibitor status to aid in future reference.

Disclosure of Interest: None declared.

PO208-TUE

Immunogenicity assessment of the first extended half-life factor VIII product, recombinant factor VIII Fc fusion protein (rFVIII-Fc), in humans

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Background: The development of inhibitors (neutralizing antibodies) to replacement factors remains a major complication in the treatment of hemophilia A. Clinical development of rFVIII-Fc included a comprehensive approach to test for the potential emergence of inhibitors and non-neutralizing antibodies (NNA).

Aims: To determine the presence of pre-existing and *de novo* antibody formation against rFVIII-Fc in previously treated patients (PTPs) with severe hemophilia A and to evaluate the longitudinal changes of NNA titers in NNA-positive subjects during the course of rFVIII-Fc therapy.

Methods: Two-hundred and thirty-three subjects in the A-LONG and Kids-A-LONG phase 3 clinical trials were tested at 3-month intervals for inhibitor development by a Nijmegen-modified Bethesda assay. The incidence of rFVIII-Fc binding antibodies was assessed by an electrochemiluminescent assay that conforms to current regulatory guidelines for detection of anti-drug antibodies (ADA). The ADA assay included a screening step, confirmation (specificity) test, titer determination, and characterization for binding to rFVIII-Fc, FVIII or Fc.

Results: No confirmed inhibitors were observed in any of the subjects. Low titer rFVIII NNA were detected in 18 subjects, either pre-existing ($n = 12$) or while on study ($n = 6$). All ADAs cross-reacted with full-length rFVIII and none were specific for the rFVIII-Fc fusion protein or the Fc domain. Individual ADA titers fluctuated over the course of the study; however, a statistically significant decline in the median ADA titers to zero was observed in both A-LONG and Kids-A-LONG after 4 to 5 months on rFVIII-Fc therapy.

Conclusion: The clinical experience to date indicates that rFVIII-Fc has a favorable immunogenicity profile in PTPs. Non-neutralizing FVIII binding antibodies were observed in approximately 8% of the study population, somewhat lower than the reported incidence of NNAs for current full length FVIII products. The substantial decline in titers after several months on study was unexpected and warrants further investigation.

Disclosure of Interest: None declared.

PO209-TUE

A study of depression in family members of hemophiliac patients

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Background: Family members of hemophiliac patients often experience stress and burden.

Aims: This study was to determine the incidence of depression in families with hemophiliac patients and associated factors.

Methods: The survey respondents were the family members of 37 hemophiliac patients who visited our hospital between September 2013 and January 2014. The survey questionnaire included questions on the general characteristics of the hemophiliac patients and the family members, and the Beck's depression inventory (BDI) scale for evaluating depression.

Results: The mean age of the family members of the patients was 41.2 years. The mother was the main caregiver for 16 patients (43.2%). The percentages of patients with severe hemophilia (<1% factor VIII, IX activity), hepatitis C virus infection, and joint disorders were 67.6%, 13.5%, and 21.6%, respectively. The mean $\pm$ SD BDI score of the family members of the patients was 6.7 ± 7.1 points, and 29.7% of the family members of the patients were found to have a tendency toward depression (≥ 10 points). The higher the severity of hemophilia in the patients, the higher the incidence of depression in their families ($P = 0.035$).

Conclusion: About 30% of the families with hemophiliac patients were found to have a tendency toward depression. The risk for depression increased with the severity of hemophilia. Family members of hemophiliac patients often experience stress and burden. Therefore, there is a need to mobilize social resources in order to share the burden of these families and to provide continuous and comprehensive care for family members.

Disclosure of Interest: None declared.

PO210-TUE

Haemtrack: UK patient home therapy reporting system

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Background: The UK Haemophilia Doctors Organisation and National Haemophilia Database (NHD) have a fully integrated software strategy, an important component of which is Haemtrack. This is a system for patients with bleeding disorders to record their home therapy.

Aims: Haemtrack was introduced to improve the timeliness and completeness of patient reported treatment records, for local and central analysis in an electronic web-based format. The system was to be easy to use, responsive and accessible to patients and medical/nursing staff for review.

Methods: Our strategy includes:- development of Android and iPhone Apps; introduction of product bar code scanning; validation and correction of data by Haemophilia Centres; automated reporting of key alert situations; and review of data with patients in clinic. Haemtrack interfaces with the UK Haemophilia Centre-based system (HCIS) and NHD to eliminate duplication of data entry. Data points include: treatment brand, dose and batch no., time/date of administration and bleed onset, reason for drug administration (prophylaxis, bleed, etc.), bleed site and severity, pain score and outcome.

Results: Haemtrack is universally adopted by Haemophilia Centres in the UK. The 2400+ patients using the system have entered over 540,000 treatment entries at 15,000 entries per month. About 80% of all UK patients with severe haemophilia A/B use Haemtrack. Clinicians are finding Haemtrack a powerful tool to interactively review with patients to optimise treatment and improve treatment compliance. Centrally, NHD are analysing Haemtrack data to better understand current treatment trends and the relative efficacy and cost-effectiveness of different regimens of prophylaxis.

Conclusion: The Haemtrack system has been extremely successful, considerably improving the compliance of patient home therapy reporting and providing a useful tool for clinicians to optimise patient treatment.

For the future, the data provided by Haemtrack will provide unique insights into potential improvements in patient care.

Disclosure of Interest: None declared.

PO211-TUE

Joint lavage followed by viscosupplementation and triamcinolone in patients with severe hemophilic arthropathy. Objective functional results

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Background: Young patients sequelaed by multiple joint bleeds waiting for the prosthetic treatment can improve function and balance of multiple joints with viscosupplementation enabling appropriate rehabilitation and the correct choice of surgical procedure of each joint. Viscosupplementation results can be improved by previous joint lavage and by adding triamcinolone.

Aims: To assess if joint lavage, viscosupplementation and triamcinolone improves force and balance in patients with severe hemophilic arthropathy.

Methods: Fourteen patients with knees hemophilic arthritis with and without involvement of other joints underwent joint lavage and subsequent injection of Hylan G-F20 and triamcinolone in all affected joints. The patients were evaluated with Neurocom[®] force and balance platforms for the following tasks: Step up and over (STP); sit to stand test (STS); one leg stance (UNI) and weight bearing squat (WBS) preoperatively, and at one and three, six and twelve months postoperatively.

Results: Sixteen knees, 15 ankles, 8 elbows and one shoulder were treated. Six patients had musculoskeletal bleeding [ankle (1), leg muscles (2) and knee (4)] at 3 months affecting results. STP showed improved lift up index for both right and left lower limbs in all moments (except at 3 months) of evaluation ($P = 0.03$ and $P = 0.02$, respectively). STS results showed that weight transfer mean (WT), rising index mean and average distance of the center of gravity to the central axis also improved significantly at 1, 6 and 12 months ($P < 0.001$, $P = 0.002$ and $P = 0.001$, respectively). One leg stance showed improved speed with eyes opened or closed in most time points but were only significant for left and right (eyes closed) and left (eyes opened) ($P = 0.02$; $P < 0.001$ and $P = 0.001$, respectively). WBS improved in all degrees of flexion (0, $P = 0.001$; 30°, $P < 0.001$; 60°, $P < 0.001$ and 90°, $P < 0.001$).

Conclusion: Joint lavage followed by injection of triamcinolone and Hylan G-F20 improves balance and force even in severe hemophilic arthropathy.

Disclosure of Interest: None declared.

PO212-TUE

Endovascular abdominal aortic aneurysm repair in a patient with mild hemophilia A on dabigatran for atrial fibrillation

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Background: Improvement in the management of hemophilia led to a significant increase in life expectancy of patients. Age-related diseases, such as cardiovascular, have increased in this population. Evidence and experience suggest that they should be treated in the same way as the general population, choosing treatment options known to be associated with low rates of bleeding complications.

Aims: Report a case of endovascular repair of abdominal aortic aneurysm in a patient with mild hemophilia A on dabigatran for atrial fibrillation.

Methods: A 78-years-old patient with mild hemophilia A (FVIII 8%) and atrial fibrillation under oral anticoagulation with dabigatran 110 mg bid (CHA2DS2-VASc 4 and HAS-BLED 3). After clinical suspicion, abdominal-pelvic angiotomography showed an infrarenal abdominal aortic aneurysm with 41 × 36 × 52 mm and an apparent flap of the *intima*, suggesting not recent limited dissection, with vascular surgery criteria.

Results: On August 2014, the patient underwent an elective endovascular graft implantation after suspension of dabigatran. A bolus of 4.000 IU of FVIII concentrate (68 kg) was infused 1 h before procedure (FVIII 108%) and in postoperative period FVIII concentrate was administered by bolus (3.000 IU 12/12 h) during 3 days and 2000 IU day⁻¹ (next 2 days), avoiding intensive treatment to prevent inhibitors (on family history, he has a brother who developed inhibitors after a hernia surgery). There were no bleeding complications related to surgery and dabigatran was introduced on day 8 after the procedure. An angiotomography performed in October showed the presence of an endoleak type II to be followed.

Conclusion: The new challenge for treatment is to provide optimal health care for this aging population. Appropriate preoperative assessment and preparation, as well as a careful perioperative multidisciplinary team prevented bleeding and thrombotic complications in this patient, opening the discussion of the use of direct oral anticoagulants in this population.

Disclosure of Interest: None declared.

PO213-TUE

Analysis of moderate phenotype of hemophilia A with novel missense mutation, W2229R in C2 domain, of which FVIII has no responsiveness with DDAVP

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Background: A male in his twenties had experienced an abnormal bleeding after tooth extraction in the past. Screening test for his bleeding tendency was performed because of requisiteness for another tooth extraction. A closer examination for prolonged APTT revealed moderate hemophilia A with 2.6% of FVIII:C. He has no family history of bleeding tendency. DDAVP was chosen as a hemostatic agent for the procedure and an infusion test was introduced. VWF:Ag and VWF:RCo elevated from 78% to 166% and from 87% to 220%, respectively, whereas FVIII:C remained to 5.3% at 2 h after administering

DDAVP ($0.4 \mu\text{g kg}^{-1}$). Recovery of rFVIII and pdFVIII/VWF products were 152% and 157%, respectively. T_{1/2} of rFVIII and pdFVIII/VWF products were 8.0 and 5.8 h, respectively. His tooth extraction was performed by administration of rFVIII product and finished without any difficulties.

Aims: To investigate a reason why FVIII did not respond with DDAVP in moderate hemophilia A.

Methods: The binding abnormality between FVIII and VWF was thought to be one of causes that DDAVP had no responsiveness. The results from ELISA to clarify the FVIII-VWF binding ability showed that VWF protein in the patient's plasma bound to FVIII from rFVIII product. F8 gene analysis was performed and revealed a novel missense mutation, W2229R (c.6742T>C, p.Trp2248Arg) in exon 25 was identified.

Results: An amino acid, W2229 located in C2 domain of FVIII protein is well known as the most critical and responsible for binding to VWF. His FVIII:Ag measured by ELISA was 2.8% and his moderate severity might be due to an increased clearance of FVIII protein unbound to VWF.

Conclusion: DDAVP is not always effective in moderate hemophilia A, meanwhile FVIII products also does not indicate its hemostatic effect in case of VWD type 2 N despite of the similar data shown in moderate to mild hemophilia A. Therefore, the efficacy of DDAVP should be confirmed in their bleeding control beforehand.

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PO214-TUE

Russian national registry of inhibitor hemophilia patients: first cut-off analysis

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Background: The number of inhibitor hemophilia patients in Russia is presently estimated 250. Due to lack of data on clinical course of disease, treatment approaches and outcomes, National Hematology Society has initiated continuously running national Registry of Inhibitor Hemophilia patients (RING).

Aims: To register clinical course of disease, treatment efficacy parameters and remote outcomes in inhibitor hemophilia A and B patients in routine clinical practice.

Methods: Data collected by physicians includes demographics, hemophilia type and severity, inhibitor titer, annual bleeding rate (ABR), comorbidities, target joints, surgeries, viral infection presence, inhibi-

tor therapy, clinical safety. Data is entered into e-database using on-line access secured by the Operator.

Results: In total 151 patients from 34 regions were included in the Registry by January 2015. Severe hemophilia is diagnosed in 90% of patients, moderate – 7%, mild – 3%. Age of inhibitors diagnosis: ≤10 years – 45%, 11–20 years – 21%, 21–50 years – 20%, ≥51 years – 6% of patients. In 35% of patients ABR was ≥16, in 38% – 6–15, in 23% – 2–5, and 4% – ≤1. Life threatening bleedings were registered in 22 patients (15%). Treatment with bypassing agents is administered in 132 (87%) patients; 11 (7%) patients undergo ITI; 9 (6%) on FVIII concentrates treatment. Bypassing agents therapy includes recombinant factor VIIa (rFVIIa) on-demand treatment – 55%, rFVIIa prophylaxis – 29%, rFVIIa and activated prothrombin complex concentrates (aPCC) on-demand – 7%, aPCC prophylaxis – 7%, aPCC on-demand – 5% of patients. Among patients on rFVIIa on-demand treatment 23 (18%) need >3 injections to stop a bleeding episode. They are identified as non-responders requiring switching to aPCC.

Conclusion: The share of non-responders to rFVIIa on-demand treatment is 18%. Collected data will contribute to improvement of patients' care and healthcare budget optimization.

Disclosure of Interest: None declared.

PO215-TUE

Septic arthritis in hemophilic target joints – a not uncommon complication – awareness is the main diagnostic feature

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Background: Septic arthritis in persons with hemophilia (PWH) is described as a rare complication with often delayed diagnosis.

Aims: To alert to a rare but important complication.

Methods: Case reports.

Results: Over the last 10 years, in our center, six PWH, median age 48 year (16–57y, 3/6 HIV positive but only 1 with impaired immunity) presented with symptoms mimicking hemarthroses but not responding to clotting factor replacement adequately despite home treatment for up to 1 week and in 2 cases even signs of additional joint bleedings, 5/6 had fever. Two patients had on demand treatment, two low dose prophylaxis (~15 IE kg⁻¹ 2–3 times week⁻¹) and two regular prophylaxis but poor adherence. Septic complications related to joint replacement are not included.

The correct diagnosis was made within 3 h after admission in all cases by combination of clinical signs and laboratory findings of highly elevated CRP and leukocytosis.

All patients underwent surgical joint lavage. Microbiological sampling before starting empiric antibiotic treatment revealed *Staph. aureus* (n = 2), *E. coli* (n = 2), *Streptococcus* and *Acinetobacter*.

Three patients developed renal and liver failure. No patient died. 4/6 patients developed severe functional joint impairments.

Conclusion: At least 5/6 of these cases of septic mono or oligo arthritis must have occurred hematogenously due to bacteremia by joint bleeding or microbleedings. Better prophylaxis might have been able to avoid those bleedings and therefore prevent septic arthritis.

The 2 cases of *Staph. aureus* might be related to insufficient disinfection in home treatment. Better hygienic training may resolve this issue. For early diagnosis of septic arthritis in PWH, it is essential to be aware of this complication in all cases of hemarthros not responding adequately to clotting factor replacement. Laboratory testing of inflammation parameters may lead to early diagnosis of septic arthritis. Any delay in diagnosis and treatment can lead to septic complications including multiorgan failure and high mortality.

Disclosure of Interest: None declared.

PO216-TUE

Managing modern major orthopaedic surgery with plasma derived FVIII concentrate (FANHDI®) in severe haemophilia A patients with advanced arthropathyLuo P-L¹, Austin S², Pink R³ and Rangarajan S⁴¹Department of Haematology, St George's Medical School;²Haematology, Guys and St Thomas' NHS Foundation Trust,London; ³Haemophilia Research Centre, Hampshire NHS Trust,Hampshire; ⁴Haematology, Hampshire NHS Foundation Trust, London, UK

Background: The routine use of plasma derived Factor VIII concentrate (Fanhdi®) in the UK has declined since 2003 following the introduction of recombinant FVIII (rFVIII) products. Nevertheless, some patients continue to use pdFVIII products due to medical indications or patient choice. This includes using pdFVIII to maintain haemostasis during surgical intervention. Whilst pdFVIII is often not a first-line option, the modern management of Patients with Haemophilia (PWH) should not limit pdFVIII usage to rescue ITI, but pdFVIII should remain an option in all settings of replacement therapy in selected groups. We describe our experience of haemostatic control in major surgery with Fanhdi®.

Aims: To assess the efficacy and safety of Fanhdi® in PWH undergoing major orthopaedic surgery.

Methods: A retrospective analysis of all patients treated with Fanhdi® from 2003–2013 in St Thomas's Hospital, undergoing major orthopaedic surgery.

Results: Twenty-six major orthopaedic procedures (10 knee replacements, 2 hip replacements, 5 ankle surgeries and 9 joint arthroscopies) were carried out on 21 patients (14 severe). The majority of procedures were covered with a FVIII infusion during the initial operative period, with conversion to bolus dosing after. The median dose (IQR) for knee replacements, hip replacements, ankle surgeries and joint arthroscopies were 586 iu kg⁻¹ (420–1010), 403 u kg⁻¹ (303–N/A), 266 iu kg⁻¹ (210–342) and 220 iu kg⁻¹ (94–277) respectively. Prior to the study, eight patients were infected with HCV and 4 were co-infected with HIV. There were no peri operative complications attributable to poor haemostasis although, as expected infection was observed in some cases. Chemical thromboprophylaxis was not used and there were no thromboembolic complications. Furthermore, none of the patients developed FVIII inhibitors in the perioperative period.

Conclusion: Our study demonstrates that Fanhdi® remains an option in modern major orthopaedic surgery being well tolerated, efficacious and safe during major orthopaedic surgery.

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PO217-TUE

Thromboelastogram for intra-operative monitoring of hemophilia patients during coronary artery bypass graft surgeryMisgav M¹, Kenet G¹, Mandelbaum T², Kassif Y³, Fruchtmann Y⁴ and Tamarin I¹¹Haemophilia & Thrombosis; ²Anesthesiology; ³Cardiovascular Surgery, Sheba Medical Center, Ramat-Gan; ⁴Pediatric Emergency Medicine, Soroka Medical Center, Bersheva, Israel

Background: Coronary artery by-pass grafting surgery (CABG) in hemophilia patients is a challenging task in particular during the intra-operative period. Monitor FVIII activity in the presence of heparin is impractical while the standard activated clotting time assay (ACT) would be inaccurate in case of low level FVIII. Thromboelastography

(TEG) is a useful tool to detect coagulopathies and was reported to be even better than ACT.

Aims: To explore the feasibility of applying TEG to monitor hemostasis and guide treatment during CABG particularly at the crucial phase of heparin titration.

Methods: Conventional ACT and Thrombelastograph (TEG 5000™) were measured simultaneously. TEG was applied using Kaolin and heparinase based cups and the following parameters were recorded (*R*, α angle, MA). Measurements were done after the first bolus of FVIII concentrate, after heparin administration ("on-pump") and after the first dose of protamine.

Results: Three patients with baseline FVIII 1–8% were operated in our center. After the first bolus of FVIII both ACT and TEG recording were normal. During heparinization ("On-pump") both ACT and TEG-R were significantly prolonged (ACT- 820s, *R*- 15.8). Following the first dose of protamine (80% of calculated) TEG-R was still longer than normal while the ACT has dropped back to baseline. At the same time TEG – heparinase has yield prolonged *R* suggesting low level FVIII activity rather than excess of heparin. Thus, a second bolus of FVIII and not protamine was administered which resulted in complete normalization of all TEG parameters. At *post-hoc* analysis we measured FVIII activity after the first dose of protamine to be only 32%.

Conclusion: The use of TEG enables goal-directed therapy with regard to FVIII, heparin and protamine administration. Adopting such an approach may result in a better outcome for patients with hemophilia going CABG surgery.

Disclosure of Interest: None declared.

PO218-TUE

Factors influencing risk of bleeding after dental extraction in hemophilia patientsMilić P¹, Bodrožić J¹, Elezović I¹, Jurišić M², Brković B², Antić D¹, Leković D¹ and Mitrović M¹¹Clinic of Hematology, Clinical Center of Serbia; ²Clinic of Oral Surgery, Faculty of Dental Medicine, Belgrade, Serbia

Background: Little is known about factors influencing bleeding risk after dental extraction in hemophilia patients (PWH).

Aims: To investigate influence of factors related to patient's characteristics and factors related to therapeutic regimen on occurrence of bleeding after tooth extraction in PWH.

Methods: Retrospective analysis of 108 episodes of tooth extractions with total 238 teeth extracted in 56 PWH in period from July 2004 until July 2014 in a single centre. Out of 108 procedures, 83 (77%) were performed in hemophilia A, and 25 (23%) in hemophilia B patients. In all patients appropriate factor concentrate or DDAVP were administered immediately before extraction providing peak level above 0.4 U mL⁻¹. Drug was administered as a single dose in 60% of extractions, while in 40% of extractions the second dose was administered 24 h later, at the discretion of treating physician. Oral antifibrinolytics were used in 92 episodes (85%). Every bleeding from extraction wound demanding additional dose of factor concentrate or DDAVP was considered as abnormal.

Results: Out of 108 episodes of tooth extraction abnormal bleeding was observed in 14 (13%). In multivariate logistic regression analysis, not use of antifibrinolytics was highly associated with increased risk of bleeding after extraction (OR = 22.4 CI 5.9–85.2). Interestingly, there was a trend to higher risk in younger patients but this factor did not reach statistical significance. Level of missing factor above 0.4 U mL⁻¹ at the time of extraction did not influence the risk. Similarly, the severity of hemophilia, bleeding phenotype, single or repeated regimen of drug administration, decreased platelet count, HCV or HIV status and numbers of teeth extracted in a single episode were not associated with risk of bleeding.

Conclusion: Except surgical risk, in patients with peak factor level above 0.4 U mL^{-1} at the time of dental extraction, not use of antifibrinolytics represents most important risk factor for occurrence of bleeding complications.

Disclosure of Interest: None declared.

PO219-TUE

Factors influencing the outcome of immune tolerance induction in patients with haemophilia A and inhibitors

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Background: Development of inhibitory antibodies to infused factor VIII (FVIII) is a serious treatment complication in previously untreated patients (PUP) with haemophilia A. Immune tolerance induction (ITI) is reported to be successful in about 60–80% of patients.

Aims: We aimed to identify parameters influencing ITI success, failure and duration in our patient cohort.

Methods: We conducted a retrospective study in haemophilia A PUPs with inhibitors who underwent a high dose (HD) ($n = 92$) or a low dose (LD) ($n = 27$) ITI protocol at the Bonn Haemophilia Care Center between 1974 and 2008. Information on inhibitor titers, pharmacokinetic data of FVIII, clotting factor usage and intercurrent bleeding were identified in our electronic patient database. Information related to hospitalizations and use of central venous devices were extracted from medical records. Statistical analysis was performed using SPSS Statistics.

Results: Data from 118 haemophilia A patients with inhibitors were available for further analysis. Seventy-five patients had high- and 43 patients had low-titer inhibitors. All 43 patients (100%) with low-titer inhibitors achieved ITI success either with the HD ($n = 23$) or with the LD ($n = 20$) ITI protocol. From 66 patients with high-titer inhibitors 49 (74.2%) achieved ITI success and 17 (25.7%) patients were ITI failures. Mean duration of all successful ITI was 788 days in high-titer inhibitor patients with regular ITI protocol ($n = 37$) and 511 days in low-titer inhibitor patients. Occurrence of ≥ 1 severe bleeding episodes during ITI had a negative influence on ITI success ($P = 0.00612$) in patients with high-titer inhibitors. Presence of a central venous device and hospitalizations had a negative influence on ITI duration ($P = 0.011$ respectively $P = 0.0015$) but not on ITI success.

Conclusion: Numbers of bleeding episodes and hospitalizations had a negative influence on ITI success and/or duration. Larger, prospective studies are needed to identify relevant predictors of ITI success and failure.

Disclosure of Interest: None declared.

PO220-TUE

Individualised treatment in haemophilia A – variability in factor VIII activity measurement for pharmacokinetic dosing

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Background: Clinical practice in most Haemophilia Centres is to use standard prophylactic regimens for patients based on weight and to adjust the dose dependent on break through bleeds. Across medicine

there is a move towards individualised therapy. Prophylaxis dosing based on individual pharmacokinetics may reduce bleeds, tissue and joint damage in Haemophilia A.

Aims: To evaluate the variability of factor VIII (FVIII) activity measurement based on one-stage FVIII and chromogenic FVIII assays for individual patients to investigate the feasibility of routine pharmacokinetic (PK) dosing in clinical practice.

Methods: Plasma from severe Haemophilia A patients on prophylaxis collected for PK studies, at 0 and 3 months was assayed for FVIII. FVIII activity assays were performed on Destiny Max coagulation analyser (StagoUK). Factor assays were performed using TriniCLOT aPTT reagent, Technoclone chromogenic assay and deficient plasma and TriniCHECK reference plasma and controls.

Results: There was no statistically significant difference in FVIII PK recovery measurements for individual patients ($P = 0.977$) between 0 and 3 months (each patient treated with their normal prophylaxis dose during this period). There was no statistically significant difference in the calculated FVIII half-life between 0 and 3 months for individual patients ($P = 0.713$). It was observed that half-life for Helixate FS < Fandhi < ReFacto AF.

Conclusion: Study has shown good correlation and recovery with associated prophylaxis dose of FVIII replacement therapy for individual patient FVIII activity measurements over time. This provides evidence that quality assured, consistent, reproducible measurement of FVIII could support PK dosing models in clinical practice.

Disclosure of Interest: J. Needham Grant/Research Support from: Award Pfizer Competitive Grant Programme, H. Lewis Grant/Research Support from: Award Pfizer Competitive Grant Programme, N. McIndoe Grant/Research Support from: Award Pfizer Competitive Grant Programme, S. Mangles Grant/Research Support from: Award Pfizer Competitive Grant Programme, S. Rangarajan Grant/Research Support from: Award Pfizer Competitive Grant Programme.

PO221-TUE

Cyclophosphamide efficacy and toxicities in the first line treatment of acquired hemophilia

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Background: Acquired hemophilia (AH) is a rare bleeding disorder. Patients develop an inhibitor against factor VIII (F8) and experience bleeding symptoms. The two goals of treatment are to control bleeding symptoms and to eradicate the inhibitor. Current recommendations suggest that cyclophosphamide (CYP) and steroids should be the first-line treatment of AH. We sought to describe our experience with this strategy.

Aims: To describe a single center experience with CYP and its toxicities in the treatment of AH.

Methods: Charts from patients presenting to a tertiary referral center from 2013 to 2014 were reviewed. Data was collected on age, comorbidities, symptoms, F8 activity, and inhibitor titers (Bethesda Units; BU). Goals of immunosuppression treatment were a BU titer <1, F8 > 50%, and resolution of bleeding symptoms.

Results: 6 patients were identified with AH, with median age = 78.5, range = 51–94; median BU titer = 57.5, range 3.1–691. 5/6 had F8 activity <1%. Four patients presented with muscular hematomas, 1 with bruising, and 1 with an intestinal bleed. 5/6 patients were treated with CYP at a median dose of 1.94 mg kg^{-1} (range 1.4–2.6 mg kg^{-1}) along with prednisone (1 mg kg^{-1}). One patient received rituximab followed by CYP. 5/6 patients received FEIBA for bleeding control. The median time to a F8 > 50% was 38.5 days (range 19–87) and the median time to a BU titer <1 was 51.5 days (range 19–92 days). 4/6 patients developed neutropenia ($n = 3$) or lymphopenia ($n = 1$). The median time to cytopenias was 48.5 days (range 43–50 days). Two

patients with neutropenia presented to the ED with fevers and required GCSF.

Conclusion: CYP with steroids resulted in remission of AH in the majority (4/6) of patients. F8 recovery occurred within 2 weeks, inhibitor eradication took twice as long and usually disappeared when cytopenias occurred. This small report underscores the need for caution with CYP. Given the risk of immunosuppression in this older population, other first-line therapies should be explored in larger trials.

Disclosure of Interest: J. Chapin Consultant for: Baxter Pharmaceuticals, S. Rutherford: None declared, M. DeSancho: None declared.

PO222-TUE

Two year use of shared guidelines and a dedicated web site to improve management of inherited bleeding disorders in the emergency department

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Background: Treatment of patients with inherited bleeding disorders (PWIBD) in the emergency department (ED) is a challenge because bleeds can be underestimated and their management not well known by ED physicians.

Aims: In 2010 a project was started involving all 8 haemophilia centres (HC) and all 44 EDs of the Region of Emilia-Romagna in north Italy to find a solution.

Methods: The project is based on guidelines for emergency treatment, education for ED staff and a dedicated web-site supporting treatment decisions and sharing data with patients' electronic clinical records.

Results: The guidelines for managing PWIBD were produced and shared by HC and ED and published on web-site www.emofiliar.it in February 2011. A web algorithm suggests the first dose of concentrates for each type and severity of bleed or trauma also tailored for PWIBD with an USB-key connected with their web-clinical record.

After training courses in each ED, the network has been active since June 2011. During 2012 and 2013 the site was visited 14,000 times, the EDs accessed the web-site 1739 times and used the algorithms 206 times (mostly for joint, muscle and mucosal bleeds). Most PWIBD treated in ED had haemophilia A (61%) followed by von Willebrand disease (21%).

We evaluated also the PWIBD management in two sample EDs: most PWIBD were male (74%) with median age of 31 years; 75% of ED access was for bleed or trauma; the time of triage-assessment and triage-treatment was reduced in 2013 vs. 2012 (27/20 and 122/90 min respectively). There was also an increase of HC medical advice in ED in 2013 vs. 2012 (54% vs. 24% cases).

Conclusion: This is the first example of a network, involving HC and ED, for managing bleeding emergencies in PWIBD with support of a web-based software. The main advantages of this system are better management of patients in ED (shorter time from triage to treatment) and improved collaboration between HC and ED. The most critical point is staff turnover in ED, with need of continual training.

Disclosure of Interest: None declared.

PO223-TUE

Evidence of APCC mode of action in a clinical study treating hemophilia A inhibitor patients with prophylaxis therapy

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Background: Hemophilia patients with inhibitors are treated either with activated prothrombin complex concentrates (APCC/FEIBA) or with activated recombinant factor VII (rFVIIa). It has been postulated that their modes of action are based on the induction of thrombin generation via complex interaction with endogenous clotting factors and platelets. Investigation of the roles of APCC protein components *in vitro* demonstrated that although prothrombin (FII) and factor Xa play key roles, other pro- and anticoagulant components are required for optimal and balanced thrombin generation.

Aims: A confirmation of the postulated mode of action of APCC, based on *in vitro* experiments, through measurement of thrombin generation assay (TGA) parameters and hemostatic factor component levels in patients.

Methods: The efficacy and safety of APCC prophylaxis compared to on-demand treatment were recently evaluated in a phase three prospective, open-label, randomized, parallel clinical study in hemophilia A patients with high titer inhibitors. Thrombin generation parameters and hemostasis factors were measured multiple times during the 12-month study. The study results were compared with *in vitro* findings to provide a biochemical explanation for the prophylactic efficacy of APCC.

Results: TGA showed significantly higher peak thrombin ($P = 0.0467$), area under the curve ($P = 0.0228$) and significantly elevated factors II ($P < 0.0001$), X ($P < 0.0001$), IX ($P = 0.0275$), VIIa ($P = 0.0265$) and protein C ($P = 0.0076$) levels were noted in the prophylaxis cohort as compared to the on-demand cohort during months 3 to 12 of APCC therapy.

Conclusion: These results support *in vitro* findings. Increased levels of pro- and anticoagulant factors were observed in patients receiving regular APCC infusion enabling the hemostatic system to respond swiftly to minor trauma and to reduce and prevent bleeds. Indeed, the bleed rate in the prophylaxis cohort was significantly lower ($P = 0.0003$) compared to the on-demand cohort.

Disclosure of Interest: K. Varadi Employee of: Baxter Innovations GmbH, S. Tangada Shareholder of: Baxter BioScience, Employee of: Baxter Healthcare, G. Schrenk Shareholder of: Baxter BioScience, Employee of: Baxter Innovations GmbH, J. Doralt Employee of: Baxter Innovations GmbH, B. Ewenstein Shareholder of: Baxter BioScience, Employee of: Baxter Healthcare, P. Turecek Shareholder of: Baxter BioScience, Employee of: Baxter Innovations AG.

PO224-TUE

A case of moderate hemophilia a with p.R1800H mutation complicated with juvenile idiopathic arthritis

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Background: Hemophilia A with the p.R1800H mutation are clinically heterogeneous, varying from mild to severe phenotype. Thirty-two patients have been reported according to the Factor VIII(FVIII) variant database and only two of the severe cases developed inhibitor. We have experienced a moderate case with inhibitor complicated with juvenile idiopathic arthritis (JIA).

Aims: **【Patient's profile】** A three-year old boy had an impaired extension of his right knee suddenly at the time of awakening. JIA and infectious arthritis were suspected. After 5 months, the prolonged APTT (59.6 s) was found at the preoperative assessment of the diagnostic arthroscopy and referred to our hospital.

Methods: He was diagnosed as a moderate hemophilia A (FVIII:C 3.8 IU dL⁻¹). Genetic analysis demonstrated p.R1800H in the FVIII molecule. Since the joint symptom occurred rapidly without any injury, it was considered atypical as a hemophilic intra-articular bleeding. To improve the impaired joint movement and make the diagnosis, synovial membrane resection was performed. The joint fluid was clear and blood was not found. The pathology of synovial membrane demonstrated the hyperplasia of the synovium cell, invasion of plasma cells and no deposition of hemosiderin, confirming JIA. Steroid was injected intra-articularly.

Results: The movement of right knee joint was remarkably improved after the surgical intervention. After 30 exposures of recombinant FVIII, the inhibitor development (1.2 BU mL⁻¹) was observed and FVIII:C was mildly decreased (1.8 IU dL⁻¹). After 2 months, the swelling of right knee joint due to the injury was observed. FVIII was administered for the purpose of both hemostasis and immune tolerance induction therapy. The joint symptom improved and the inhibitor disappeared.

Conclusion: We experienced a case of moderate hemophilia A with p.R1800H complicated with JIA. The danger signal caused by tissue damage by the surgery in addition to the JIA-related impaired immune system might be the risk factors of the inhibitor.

Disclosure of Interest: None declared.

PO225-TUE

Long term follow-up in the use of different types of central venous catheters in children with hemophilia on prophylaxis or immune-tolerance regimen

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Background: Among hemophiliac children, sometimes peripheral venous access may not be able to support regular treatment; therefore an alternative way of administering concentrates is required, such as central venous catheters (CVCs). CVCs can be externally tunneled (like Broviac) or completely implantable (like Port).

Aims: Aim of the study is to compare the two devices in terms of complications during the long-term follow-up.

Methods: Hemophiliac children who underwent implantation of CVC from 2005 to 2014 at the Hemophilia Center of Padua were retrospectively evaluated for collecting information according to the two types of CVCs, patients' characteristics and complications during follow-up.

Results: Twenty-two CVCs were implanted in 12 children, among which 12 were Broviac and 10 Port. The median number of CVCs implanted per patient was 1.5 (range 1–3) for Broviac and 1 (1–2) for Port. Six patients required one CVC (4 Port and 2 Broviac), while the other six patients needed two or more. Mean age for implantation and medium time of observation were not statistically different in the two groups. When using Broviac, we had a total of 1.43 complications in 1000 CVC days and 0.83 complications/1000 led to the removal of the device (6 dislocation, 1 infection); the use of Port caused a total of 0.93 complications/1000 CVC and 0.55/1000 resulted in the removal of the device (2 infection, 1 hematoma into the pocket). Complications handled conservatively were 4 external ruptures for Broviac, one infection for Port and one occlusion in both groups. No significant statistical differences were observed between the two types of CVCs considering the total of complications. Mechanical complications were more frequently related to Broviac ($P < 0.001$).

Conclusion: CVC is an indispensable tool for hemophilia treatment though adverse events are not rare. The choice between the two types of CVCs should be based on careful analysis of the reasons for using CVC, age of child, preferences and ability to manage the device by the family.

Disclosure of Interest: None declared.

PO226-TUE

Successful multi-vessel percutaneous coronary intervention for acute coronary syndrome in a severe Taiwanese hemophiliac with HIV infection under HARRT: the first reported case in Chinese population

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Background: Most of the limited studies on acute coronary syndrome (ACS) of patients with hemophilia (PWH) came from the West countries, and experience originated from Asian is few.

Aims: We report the first case of Taiwanese PWH with ACS, receiving successful multi-vessel percutaneous coronary intervention (PCI) with bare metal stents (BMS).

Methods: A 56-year-old Taiwanese with severe-type hemophilia A had his past history of his hypertension, NIDDM, hemophilic arthropathy of his knees and ankles, and renal stones. He had HIV infection under HARRT and smoked 20 cigarettes day⁻¹ for 30 years.

Results: Angina happened to him in the early 2012, and he was advised of taking Aspirin 100 mg daily. Since July 2013, angina and dyspnea had sometimes occurred. Thallium 201 myocardial perfusion scan revealed severe myocardial ischemia with transient ischemic dilatation sign. However, he had been refusing treatment, except Aspirin-taking. He finally received PCI via right radial artery access due to terrible angina on 25 December, 2014. Before PCI, a loading dose of 8000 IU heparin with Clopidogrel 300 mg and Aspirin 300 mg was prescribed; Meanwhile, the FVIII trough level was kept $\geq 80\%$ for the following 48 h. Totally BMS were deployed over the middle and distal segment of left circumflex coronary artery, the ostium of left anterior descending artery, the posterior-lateral branch and proximal segment of right coronary artery. After PCI, Clopidogrel 75 mg with Aspirin 100 mg once daily for 2 months were planned, with keeping the FVIII trough level $\geq 20\%$. No complication was found. However, gross hematuria once and intramuscular hemorrhage once happened during dual antiplatelet therapy, and no more bleeding episode occurred after adjusting FVIII trough level to $\geq 25\%$.

Conclusion: Under the support of FVIII replacement, PWH could tolerate peri-PCI procedures and medications safely. FVIII trough level might need to be adjusted during dual antiplatelet therapy if the patient has extra underlying predisposing factors for bleeding.

Disclosure of Interest: None declared.

PO227-TUE

Relationship between quality of life and coagulation factor level in hemophilia carriers: preliminary resultsYoung J¹, Grabell J², Rydz N³, Hopman W², Good D⁴, Mahlangu J⁵ and James P²¹Pathology & Molecular Medicine, Queen's University; ²Kingston General Hospital, Kingston; ³Foothills Medical Centre, Calgary; ⁴Queen's University, Kingston, Canada; ⁵Johannesburg Hospital, Johannesburg, South Africa

Background: Hemophilia A and B are X-linked deficiencies of Factor VIII (FVIII) and IX (FIX) respectively. Males are affected, while females are carriers of the disease. Recently, it has become clear that more than a third of hemophilia carriers (HC) are symptomatic. Carriers may experience prolonged bleeding postpartum, following trauma and surgery as well as menorrhagia. To date, this is the first research to incorporate both a bleeding assessment tool, to assist in objectively quantifying severity of bleeding symptoms, as well as a validated quality of life questionnaire to facilitate an understanding of the relationship between symptoms and QoL.

Aims: Therefore, the objective for this study is to measure quality of life (QoL) of HC and investigate its relationship to bleeding severity and coagulation factor level FVIII/FIX (FL).

Methods: This is a multi-centre, prospective observational study. Eligible subjects are female carriers of Hemophilia A or B, >18 years of age. Subjects are excluded if they have another potential cause of bleeding. A blood sample was obtained for coagulation factor testing and FVIII or FIX genotyping. Subjects completed the SF-36v2 quality of life questionnaire to measure QoL, and a Self-administered bleeding assessment tool (Self-BAT) to measure bleeding severity.

Results: To date, 28 HC have been enrolled (20 Hemophilia A, 8 Hemophilia B) with mean age 41 years (range 21–64), mean FL 0.56 (0.15–1.24), mean BS 9 (0–28). Preliminary analysis shows a significant positive correlation between QoL and FL ($r = 0.411$, $P = 0.030$) and a significant negative correlation between QoL and BS ($r = 0.466$, $P = 0.013$).

Conclusion: In summary, these data suggests that the QoL of hemophilia carriers is significantly associated with FL and corresponding severity of bleeding symptoms.

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PO228-TUE

Does quality of treatment influence global hemostasis irrespectively of FVIII levels in patients with hemophilia A?Berndtsson M¹, Zetterberg E², Holmström M³, Mahmoud Hourani Soutari N¹, Mikovic D⁴, Elezovic I⁵ and Antovic J⁶¹Department for Clinical Chemistry, Karolinska University Hospital, Stockholm; ²Department of Translational Medicine, Lund University, Malmö; ³Coagulation Unit, Haematology Centre, Karolinska University Hospital, Stockholm, Sweden; ⁴Haemostasis Department and Haemophilia Centre, Blood Transfusion Institute of Serbia; ⁵Clinical Centre of Serbia & Faculty of Medicine, University of Belgrade, Belgrade, Serbia; ⁶Institute for Molecular Medicine and Surgery and Department of Clinical Chemistry, Karolinska Institute and Karolinska University Hospital, Stockholm, Sweden

Background: Patients with severe hemophilia A (hA) on prophylaxis have better joint status than patients treated on-demand. In Sweden, also patients with mild/moderate hA get adequate factor replacement treatment in contrast to many other countries. Although it is generally accepted that both global hemostasis (GH) and clinical status depend primarily on FVIII level, some discrepancies has been observed.

Aims: To study GH in hA patients with different access to FVIII concentrate in Sweden and Serbia.

Methods: GH was examined in 19 hA patients treated in Sweden (severely affected on prophylaxis) and 19 patients matched by disease severity treated in Serbia (severely affected treated on-demand, others less adequately treated). Thirteen patients from each group were matched according to their FVIII level and evaluated separately.

The study was approved by local medical ethics committees.

Thrombin generation was determined using the chromogenic ETP and the fluorogenic CAT assays. OHP and fibrin network porosity were examined using previously described in-house methods.

Results: The groups of patients matched according to FVIII level had similar FVIII results; Swedish median 0.06 kIE L⁻¹, SD 0.1; Serbian median 0.062 kIE L⁻¹, SD 0.085.

Swedish patients had higher OHP ($P < 0.0001$) and ETP ($P = 0.03$) compared to Serbian patients. After matching for FVIII level, OHP differed, although insignificantly between the groups ($P = 0.1$), while ETP was similar.

Clot permeability was higher in Serbian patients compared to patients from Sweden ($P = 0.02$). This difference was borderline statistically significant in patients matched according to FVIII level ($P = 0.06$).

Conclusion: Our results suggest that quality of the treatment may contribute to global hemostatic capacity in patients with hA.

It seems that the generation of fibrin and the quality of fibrin structure is less dependent on FVIII levels compared to thrombin generation. To understand the potential underlying mechanisms, more patients matched for FVIII levels need to be included in future studies.

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PO229-TUE

Observational Immune Tolerance Induction Research Program (OBSITI) – a multifaceted approach to explore immune tolerance induction

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Background: ObsITI is an international, open-label, uncontrolled multicenter observational program initiated in December 2005. The study admits hemophilia A (HA) patients of any age and with any severity, with a confirmed inhibitor titer ≥ 0.6 BU, and reduced FVIII recovery and/or FVIII half-life. Patients with risk factors historically associated with a poor ITI prognosis as well as good prognosis are included. Patients are treated preferably according to the Bonn protocol.

Aims: The aim of the program is to evaluate patient and therapy related variables on ITI course, outcome and morbidity in HA patients.

Methods: ObsITI has several satellite studies which additionally look at other factors related to tolerization: the thrombin generation sub-study evaluates the correlation between the clinical bleeding phenotype and the patient's thrombin generation capacity before and during ITI, the sub-study about immunogenotyping investigates the impact of genetic factors on the ITI outcome, the concentrate-based thrombin generation assay (TGA) as well as the characterization of specific FVIII epitopes and the immunological sub-study look into specific aspects for personalizing ITI treatment and predicting the outcome. The results obtained from the sub-studies will be correlated with the ITI success rates.

Results: As of February 2015, a total of 151 patients from 16 countries have been documented for ObsITI. Another 6 countries are in IRB process. One hundred and Fourteen patients completed the study, 27 patients discontinued and 38 patients are ongoing. Preliminary result show a significant correlation between the bleeding rate during ITI, the peak titre during ITI, the inhibitor titre at start of ITI >10 BU and the number of poor prognosis factors with ITI outcome.

Conclusion: ObsITI is the largest prospective ongoing study on ITI with the potential to extend the knowledge on ITI as well as to tailor ITI treatment to each patient.

Disclosure of Interest: None declared.

PO230-TUE

Defining adherence to prophylaxis in haemophilia: results of a delphi consensus procedure

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Background: Adherence to prophylaxis is mostly defined as administration of 80% of prescribed medication. Recently, the importance of other aspects of adherence, such as timing, dosing planning, and communication has been underlined. However, a broadly accepted definition is lacking.

Aims: To develop expert consensus on what is regarded as adherence to prophylaxis in haemophilia.

Methods: Using the validated Delphi method, a systematic consensus procedure was performed in three rounds. Firstly, all aspects of adherence to prophylaxis were generated and clustered into themes. Secondly, the main themes were identified and prioritized. Thirdly, different combinations of themes and eight clinical cases were labelled as either adherent, sub-optimally adherent or non-adherent. Consensus was considered reached if at least 80% of the experts agreed.

Results: Twenty three Dutch haemophilia professionals and patients participated. Consensus was reached (agreement $>84\%$) on the final definition of adherence to prophylaxis. Percentage of missed infusions, percentage of changes in dosage (IU) and percentage of infusions not administered at prescribed time point were considered the most important aspects of adherence to prophylaxis. This resulted in three adherence categories: *adherent*: missing $<15\%$ of infusions, $<10\%$ dose (IU) changes and $<30\%$ deviation of infusions with regard to prescribed time point; *sub-optimally adherent*: missing 15–25% of infusions, $<25\%$ dose changes or $>30\%$ deviation of dosing from prescribed time point; *non-adherent*: $>25\%$ missing or $>25\%$ dose changes.

Conclusion: This study underlines that adherence as experienced by haemophilia professionals is complex but can be described by a set of three treatment aspects: missed infusions (%), dose changes (%) and infusions not administered at prescribed time point (%). This definition is simple and can be easily applied to describe adherence to prophylaxis in research and clinical care.

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PO231-TUE

Utilization predictability for BAX 855 vs. BIIB031: a descriptive comparison from pivotal trials

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Background: Current treatment for Hemophilia A includes prophylaxis with factor VIII (FVIII) infusions every other day and up to every 3rd day to reduce the number of hemorrhages. New treatment options include FVIII concentrates with extended pharmacokinetic half-lives. Treatment with these products will prompt changes to existing treatment patterns. To understand the value these new products provide, efficacy and product utilization should be compared.

Aims: To compare annualized bleeding rates (ABR) and utilization for two extended half-life FVIII products based on data from their pivotal Phase III clinical trials and discuss implications on real-world use.

Methods: Comparable data on ABR and utilization for prophylaxis were extracted for BIIB031 (Biogen Idec) and BAX 855 (Baxter) from two pivotal trials. Median (IQR) ABR, prophylaxis dosing regimen, mean weekly utilization (standard deviation) and the dosing minimum and maximum range were descriptively compared. Data for BIIB031 was from the individualized prophylaxis arm in the pivotal trial and data for BAX 855 was from the prophylaxis arm from the pivotal trial.

Results: The median (IQR) ABR in the BIIB031 individualized prophylaxis arm in the pivotal trial is 1.6 (0.0; 4.7). The dosing was 25–65 IU kg⁻¹ every 3–5 days. The mean weekly dose (standard deviation) and minimum and maximum range in IU kg⁻¹ were 85.4 (19.3), min: 54.0, and max: 141.5. The median (IQR) ABR in BAX 855 prophylaxis arm in the pivotal trial is 1.9 (0, 5.8). The dosing was 45 $\pm$ 5 IU kg⁻¹ twice weekly. The mean weekly dose (standard deviation)

tion) and minimum and maximum range in IU kg⁻¹ were 85.7 (7.6), min: 67.1, and max: 114.9.

Conclusion: The efficacy from both studies demonstrates similar results with both products; however, the dosing regimen and weekly utilization distribution with BAX 855 has a smaller variance. This data suggests that BAX 855 may provide similar outcomes with greater utilization predictability for payers compared to BIIB031.

Disclosure of Interest: None declared.

PO232-TUE

Origin of mutation and mosaicism in families with sporadic cases of hemophilia A in Sweden

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Background: In about half of newly diagnosed cases of Swedish hemophilia A (HA) the proband is a sporadic case, i.e. there is no previous history of hemophilia in the family. Some of the genotypically non-carrier mothers have later gone on to have two descendants with the same mutation, maybe due to germline mosaicism.

Aims: To define the origin of mutation in sporadic cases of HA, reveal possible sex-specific differences in mutagenesis and find somatic mosaics among non-carrier mothers.

Methods: Blood was collected from relatives in three generations in 45 families with sporadic severe hemophilia retrieved from the Malmö Hemophilia Center's register. Mutations were characterized and microsatellite haplotyping determined whether the X-chromosome carrying a *de novo* mutation was of grandpaternal or -maternal origin. Digital polymerase chain reaction (PCR) is being used to reveal mutations not found on conventional sequencing in order to obtain evidence of somatic mosaicism in non-carrier mothers. The participants gave informed consent.

Results: In 28/45 families the mother carried the mutation. In five of these families the grandmother was also a carrier resulting in 82% (23/28) of the mothers having a *de novo* mutation. The X-chromosome with the *F8* mutation was of paternal origin in 16/23 (70%) cases and of maternal origin in 4/23 (17%), whereas 3/23 (13%) cases were uninformative. The mutation types were gender correlated as follows: inversions: paternal: 8/16 and maternal: 0/4; frameshift (insertion/deletion): paternal 4/16 and maternal 2/4; and point mutations: paternal 4/16 and maternal 2/4. In 17/45 families the mother of the sporadic case was a non-carrier, as determined by conventional sequencing; analyses of potential mosaicism with digital-PCR are ongoing.

Conclusion: In 40/45 the sporadic case results from a mutation in the last two generations, indicating a low carrier risk in relatives. In 82% (23/28) the mothers had a *de novo* mutation where the X-chromosome was of paternal origin in 70% (16/23).

Disclosure of Interest: None declared.

PO233-TUE

Differences in the trough levels of factor VIII depending on blood groups

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Background: An important factor of the efficacy of prophylaxis is the length of time for which an individual has a low coagulation factor activity. This period is more dependent on the half-life of the drug and

the frequency of infusion than on *in vivo* recovery and the amount of drug infused. Therefore, for maintaining effective prophylaxis, determination of the factor that affects the half-life of the drug is very important.

Aims: The half-life of FVIII is markedly influenced by von Willebrand factor (VWF). We evaluated the differences in trough levels of FVIII:C between patients with blood group O who has relatively low levels of VWF antigen and those with blood groups non-O.

Methods: We included 23 patients with severe hemophilia A (FVIII:C < 1%) on primary and secondary prophylaxis. Patients with a history of having inhibitors, patients using a central venous catheter for infusion and patients using factor VIII preparations containing VWF were excluded. We measured FVIII:C after 2 days of infusion with no bleeding in the most recent data on December 31, 2014. Then, we compared the trough levels of FVIII in patients with O or non-O.

Results: Eight patients had blood group O and 15 were non-O. No significant differences were observed in the ages in the two groups (O:17.4 ± 11.4 years vs. non-O:15.8 ± 13.0 years, *P* = 0.76). No significant difference was observed in the dose of factor VIII per body weight between the two groups (O:26.8 ± 6.0 IU kg⁻¹ vs. non-O:25.0 ± 9.2 IU kg⁻¹, *P* = 0.58). The trough level of FVIII:C in the O group was lower than that in the non-O group (O:2.4 ± 1.6% vs. non-O:4.4 ± 2.5%, *P* = 0.03).

Conclusion: Although a significant difference was not observed in the backgrounds between the two groups, the trough level of FVIII:C was significantly lower in the O group than in the non-O group. Thus, our result suggests that the half-life of FVIII:C is short in patients with the O blood group. Therefore, it seems to be important to consider blood type in planning the prophylactic regimen to the patients with hemophilia A.

Disclosure of Interest: None declared.

PO234-TUE

Continuous infusion of FVIII in obese patients with severe haemophilia A: is weight-based dose-adjustment required?

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Background: The prevalence of obesity is increasing in the western world, and so is in patients with haemophilia. A question has been posed if the dosing of clotting factor in haemophilia patients should be adjusted for obesity. The standard formula for clotting factor concentrations uses factor VIII (FVIII) recovery value of 2.0 IU dL⁻¹/IU kg⁻¹. However, recent studies proved significant variations in FVIII recovery depending on the patient's body weight (BW). To date, no study investigated the impact of BW on the dosing of factor concentrate for continuous infusion (CI).

Aims: Here we present a case of obese haemophilia patient that underwent total knee replacement covered by a CI of FVIII concentrate.

Methods: A 48-years old patient with severe haemophilia and morbid obesity had a total knee arthroplasty in February 2014. The patients BW was 150 kg, height 177 cm and BMI 47.9 kg m⁻². The procedure was covered by concentrate of a second generation recombinant FVIII given in CI through a central venous catheter.

Results: We easily achieved the aimed haemostatic levels of FVIII by having FVIII concentrate bolus dose and CI rate calculated based on the BW of 100 kg. The FVIII levels were maintained between 90% and 128% at all times, and the rate of CI had to be adjusted only once. The surgery procedure and the early postoperative period went uneventful, and the patient was discharged on day 11.

Conclusion: The present case describes a successful use of weight-adjusted approach to the calculation of FVIII concentrate dosage for the CI in obese haemophilia patient.

Disclosure of Interest: None declared.

PO235-TUE

Use of APCC in combination with tranexamic acid: a single centre experience

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Background: Antifibrinolytic agents are not recommended to be used in concomitance with activated prothrombin complex concentrate (FEIBA). Nevertheless, it is discussed whether adjunct tranexamic acid (TXA) may improve haemostasis and be safe. In fact, some ex vivo evidence has shown that adjunct TXA does not increase the endogenous thrombin potential but normalizes clot stability. In our centre concomitant use of clotting factor concentrate and TXA is a standard of care in patients with inherited bleeding disorders who suffer mucosal bleeds or undergoing invasive procedures on mucosa membranes (e.g. dental extraction).

Aims: To evaluate the safety and the effectiveness of TXA as a junctive therapy to FEIBA.

Methods: A retrospective review of patient charts were used to evaluate the concomitant use of FEIBA and TXA.

Results: Nine patients underwent 16 treatment courses with FEIBA and tranexamic acid at the age of 19 to 70 years (median: 48 years). The reason of treatment was single tooth extraction (4 patients), multiple teeth extractions (8 patients), one severe nose bleeding, one severe oral cavity bleeding (palate), one intensive bleeding in oral cavity and posterior wall of nasopharynx, one upper GI tract bleeding. The concomitant treatment lasted 3 to 14 days (median: 5 days), TXA treatment continued for overall 6 to 14 days (median: 7 days). FEIBA was administered at a median daily dose of 152.2 U kg⁻¹ (min-max range: 72.7–179.0 U kg⁻¹). TXA was administered at the dose of 1.0 g every 8 h orally in all patients. All treatment courses were successful without any adverse event including thrombosis.

Conclusion: This small case series showed that the concomitant use of systemic TXA with FEIBA was effective and safe. Larger cohort of patients is required to support these findings and a risk-benefit evaluation.

Disclosure of Interest: None declared.

PO236-TUE

Non-neutralizing antibodies modulate factor VIII pharmacokinetic in patients with hemophilia A without FVIII inhibitors

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Background: Prophylaxis with factor VIII (FVIII) products is the current gold standard for treating patients with hemophilia A. Treatment dose and frequency depend on pharmacokinetic parameters such as FVIII half-life, which differs significantly among subjects. Circulating von Willebrand factor (VWF) level is a major determinant of FVIII half-life, but only partially explains the observed variability, thus raising the question of which other parameters are important.

Aims: To evaluate the influence of non-neutralizing, anti-FVIII IgG antibodies on FVIII half-life.

Methods: FVIII half-life was determined in 42 adult, regularly treated hemophilia A subjects without a history of FVIII inhibitors (Kepa et al. Haemophilia 2015). Baseline plasma samples were analyzed for anti-FVIII IgG antibodies using ELISA assays (Whelan et al. Blood 2013). Apparent affinity (K_A) of anti-FVIII antibodies was assessed by competition-based affinity-ELISA when IgG titers were $\geq 1:40$ (Hofbauer et al. Blood 2015). The effect of anti-FVIII IgG antibodies on FVIII half-life, independent of confirmed predictors age and VWF antigen, was investigated by multiple linear regression.

Results: Nine subjects had anti-FVIII IgG antibodies with titers $\geq 1:40$, predominantly IgG1 and IgG3 ($K_A \leq 3.8 \times 10^9 \text{ M}^{-1}$). IgG2 ($K_A = 1.1 \times 10^9 \text{ M}^{-1}$) and IgG4 ($K_A = 2.3 \times 10^{11} \text{ M}^{-1}$) were observed in one subject only. The effect of anti-FVIII IgG antibodies with a titer $\geq 1:40$ on FVIII half-life was significant ($P = 0.03$), even after adjusting for age and VWF antigen level. Median FVIII half-life was 8.2 h (IQR 7.5–9.3) in patients with anti-FVIII IgG antibody titers $\geq 1:40$ and 10.2 h (IQR 8.8–13.2) in patients negative for anti-FVIII IgG antibodies.

Conclusion: Our data demonstrate that non-neutralizing, anti-FVIII IgG antibodies are an additional independent contributor to variations in FVIII half-life. We therefore suggest that screening for anti-FVIII IgG antibodies could be beneficial in tailoring FVIII prophylactic regimens for hemophilia A patients.

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PO237-TUE

IPAQ scoring and carotid intima media thickness in patients with haemophilia

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Background: The hemophilia patients have the problems of elderly people nowadays, because they are aging as a result of effective usage of factor concentrates. Logically, hypocoagulability in these patients may have preventative role against acute complications of atherosclerotic plaque rupture but not preventive for developing and progression of atherosclerotic plaques.

Aims: The study aimed to determine Carotid intima-media thickness (CINT) values as an early marker of atherosclerosis and its possible relationship between sedentary life style which quantified with International physical activity questionnaire (IPAQ) score in these patients.

Methods: Haemophilia patients were recruited from the Ege Hemophilia Center. The study was approved by the Ege University ethics committees and inclusion took place after written consent had been obtained. Hemophilia patients were eligible for inclusion regardless of the severity of Hemophilia. CINT measurements were obtained with high resolution B-mode ultrasound transducer. A Turkish version of IPAQ was filled by the patients. The physical activity score was calculated as metabolic equivalent (MET)-minutes week⁻¹. SPSS version 20 was used for the statistical analysis and the correlation between the CINT and physical activity score and physical activity category was evaluated by using Pearson and Mann-Whitney *U* test respectively. *P* value being <0.05 was considered statistically significant.

Results: A total of 66 hemophilia patients (49 hemophilia A, 17 hemophilia B) were included in the study. Mean age of the patients was 37.5 ± 11.6 . Mean CINT in hemophilia patients were 0.68 mm. Mean activity score was 816 MET-min/w and 44 patients had low, 14

patients had moderate and 7 patients had high activity. CIMENT value and IPAQ score had negative correlations ($P < 0.05$).

Conclusion: To our knowledge there is not a study evaluating the physical activity score of hemophilic patients and correlation between these values. In our study we found that a negative correlation between IPAQ score and CIMENT.

Disclosure of Interest: None declared.

PO238-TUE

Left ventricle diastolic functions and physical activity score in adult hemophilia patients: aegian haemophilia center experience

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Background: The E/A ratio is the ratio of the early (E) to late (A) LV filling velocities. The mitral E/A ratio is a echocardiographic marker of the function of the left ventricle (LV). An abnormality in the E/A ratio indicates that LV cannot fill with blood properly in the diastole. In a healthy heart, the E velocity is greater than the A velocity. In certain disease processes and with aging, the walls of LV can become stiff, increasing the back pressure as it fills, which slows the early filling velocity, thus lowering the E/A ratio.

Aims: We aimed to determine LV diastolic dysfunction and physical activity relationship in hemophilia patients.

Methods: Hemophilia patients were eligible for inclusion regardless of the severity of Hemophilia. A Turkish version of International physical activity questionnaire (IPAQ) was filled by the patients. The physical activity score was calculated as metabolic equivalent (MET)-minutes/week and classified in three levels. The E/A ratio was measured by placing sample volume of pulse wave Doppler tracer across the mitral inflow. The reversal E/A shows impaired relaxation. SPSS ver. 20 was used for the statistical analysis and the correlation between E/A ratio and physical activity score was researched by using Spearman's test. P value being <0.05 was considered statistically significant.

Results: A total of 66 hemophilia patients were included in the study. Mean age of the patients was 37.5 ± 11.6 . Mean activity score was 816 MET-min/w and 44 patients had low, 14 patients had moderate and 7 patients had high activity. Mean E/A ratio was 1.26 and there was a positive correlation between E/A ratio and IPAQ score ($P < 0.05$).

Conclusion: Sedentary status due to chronic joint problems and bleeding diathesis in hemophilia patients may have negative effects on diastolic functions of LV. Regular aerobic exercise programs may improve the diastolic functions and may prevent from diastolic heart failure as the destructive result of the diastolic dysfunction.

Disclosure of Interest: None declared.

PO239-TUE

Regional data supports need for uniform approach to Extended Half-Life (EHL) product transition

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Background: Extended half-life factor VIII and IX products differ in pharmacokinetic (PK) profiles, but both offer the potential for

improved factor coverage with less frequent dosing. Data guiding individualized approaches for transition to EHL products are lacking.

Aims: To examine "real-world" experience transitioning patients to EHL FVIII and FIX products including use of PK data and dosing selection.

Methods: We retrospectively reviewed EHL factor transition practices from 3 region I HTC's, including patient age, PK data (timed levels, and where possible, estimated terminal half-life ($t_{1/2}$) by first order decay) and dosing strategy.

Results: Thirteen patients transitioned to an EHL product ($N = 159$ severe hemophilia patients), 8 Eloclate, 5 Alprolix. Age range was 3–58 year. All patients had ≥ 2 post-dose factor levels; 5 had PK data from a standard factor product as well. Median Eloclate $t_{1/2}$ was 14.6 h (range 10.8–23.8). Alprolix activity was 2–15% at day 7. One center used the no-cost central reference lab in comparison with local lab; no significant variance was noted. Dosing strategies for Eloclate varied widely (range 80 U kg⁻¹ q72 h– 25 U kg⁻¹ q96 h). Alprolix was dosed at 50 IU kg⁻¹ weekly. Four of 8 Eloclate patients returned to their original factor for reasons of minimal $t_{1/2}$ extension, breakthrough bleeds, or insurance issues. Of the remaining 9 patients on EHL products, 3 maintained a standard factor product for breakthrough bleeding.

Conclusion: The wide variation in $t_{1/2}$ and dosing strategies for EHL factors highlights an imperative to obtain at least minimal PK data to compare current factor to EHL factor within individuals. This could be done at a central lab if inconvenient to come to HTC labs. A consistent approach to sparse PK sampling from patient to patient and center to center both informs tailored prophylaxis approaches and allows meaningful comparisons across centers. This presents a ready opportunity for regional HTC collaboration as well.

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PO240-TUE

DDAVP-induced rise in pre-infusion von willebrand factor levels may slightly affect the pharmacokinetics of plasma-derived factor VIII concentrate in severe hemophilia A patients

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Background: The variability in half-life and mean residence time (MRT) of factor VIII (FVIII) concentrates is strongly associated with pre-infusion von Willebrand Factor (vWF) levels. We hypothesized that half-life and MRT of FVIII can be improved by increasing the pre-infusion endogenous vWF level through administration of 1-deamino-8-D-arginine vasopressin (DDAVP).

Aims: To evaluate the effect of raising plasma levels of vWF (by administration of DDAVP) prior to infusion of FVIII concentrate on the pharmacokinetics of FVIII in severe hemophilia A patients.

Methods: In this randomised, cross-over, placebo controlled study four severe hemophilia A patients were included. Each patient received two administrations of 50 IU kg⁻¹ plasma-derived FVIII concentrate, preceded one hour by either intravenous DDAVP or placebo. Pharmacokinetic

kinetic parameters were calculated by model independent methods using the plasma FVIII levels before and at 10 and 30 minutes and 1, 3, 6, 10, 24, 32 and 48 h following FVIII administration.

Results: In all four patients vWF increased significantly 1 h after DDAVP administration (mean respectively 1.35 to 2.69 IU mL⁻¹), whereas vWF remained stable after placebo infusion. The MRT of FVIII concentrate increased in all patients (mean from 17.6 h to 19.9 h, $P = 0.07$; 95% CI for MRT change: +4.7 to -0.3 h). Following administration of DDAVP there was a tendency towards a lower recovery of FVIII and an increase in its reciprocal equivalent, the volume of distribution. These differences did not reach statistical significance.

Conclusion: These results suggest that a DDAVP-induced rise in pre-infusion vWF levels may slightly affect the pharmacokinetics of plasma-derived factor VIII concentrate in severe hemophilia A patients.

Disclosure of Interest: None declared.

PO241-TUE

Population pharmacokinetic model of recombinant single-chain factor VIII (rVIII singlechain) in patients with hemophilia A

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Background: rVIII-SingleChain, a novel recombinant Factor VIII (FVIII), is a single-chain construct in which a truncated B-domain covalently links the heavy and light chain.

Aims: As part of the clinical development of rVIII-SingleChain, a population pharmacokinetic (PPK) analysis was undertaken, utilizing data in adult and adolescent patients with hemophilia A in order to characterize the pharmacokinetics (PK) of rVIII-SingleChain at a population level.

Methods: A nonlinear mixed effects modeling approach (NONMEM) was used to analyze data from Study CSL627_1001. Blood PK samples from 72 patients (12 to 65 years) were collected to determine plasma FVIII activity using a validated chromogenic assay. The effect of various covariates in the PPK model was tested. Visual predictive check (VPC) was used for model evaluation.

Results: A 2-compartmental model with first-order elimination adequately described the FVIII plasma activity data following rVIII-SingleChain dosing. Body weight and age were found to be significant covariates on both clearance and central distribution volume. The VPC results implied that the model was stable and the parameters were estimated with good precision. Using the final PPK model, simulations predicted that rVIII-SingleChain dosing at 40–50 IU kg⁻¹ every 3 days dosing would result in steady-state trough FVIII levels above 1% in about 90% of patients, and dosing at 50 IU kg⁻¹ twice a week at would achieve at least 1% trough levels in 73–90% of patients.

Conclusion: The PPK model adequately characterized rVIII-SingleChain PK and the model can be utilized as a tool to simulate FVIII activity-time profiles for various dosing scenarios.

Disclosure of Interest: Y. Zhang Employee of: CSL Behring, T. Limsakun Employee of: CSL Behring, D. Bensen-Kennedy Employee of: CSL Behring, A. Veldman Employee of: CSL Behring GmbH, J. Sidhu Employee of: CSL.

PO242-TUE

Discrepancy between the factor VIII levels and severity of hemophilia A in two maternal cousins' patients

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Background: What's known about hemophilia is that all males affected have the same severity as other affected males in the same family which is the result of the inheritance of the same mutation. However, other additional mutations can occur in some patients leading to different factor levels among hemophiliacs belonging to the same family.

Aims: to report a rare case of a family with two hemophiliacs with different factor levels and to discuss the different inheritance patterns.

Methods: The first patient is 27 years old, was diagnosed with severe hemophilia A, he was the only hemophiliac in the family, until the birth of his maternal cousin who has moderate hemophilia (factor VIII1.8%).

The family tree shows that their mothers are half-sisters by their father.

The patients' grandfather has married his wife who has familial relationships with him; their mothers are paternal cousins. They gave birth to a boy and a girl who is the mother of the first patient.

The grandfather has married another wife who has also familial relationships with him; they are paternal cousins, they gave birth to four girls, one of them is the mother of the second patient. No other hemophiliac in this family. Molecular genetic testing will be performed to identify the mutations.

Results: No similar case reported in the literature. the inheritance patterns discussed:

A germinal mosaic in the grandfather's gametes. Mutated spermatozoons were transmitted to both half-sisters who transmitted the mutation to their sons and the presence of additional mutations leading to different factor levels.

Both mothers are carriers and each one has a different mutation and a different hemophilia severity.

Two sporadic cases: Due to the intermarriages, there is a complex additional mutations transmitted producing different factor levels in the two affected boys.

Conclusion: The genetic testing (of the patients, their mothers and their maternal grandfather) is the only test which will decide on the mode of inheritance of hemophilia in this family.

Disclosure of Interest: None declared.

PO243-TUE

The ahead study: effectiveness interim results after one year of observation, an analysis of the European patients cohort

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Background: The ADVATE Haemophilia A Outcome Database (AHEAD) study captures long-term outcome data on patients with haemophilia A (HA) receiving rAHF (ADVATE) treatment under routine clinical practice.

Aims: We report effectiveness interim results from European countries (without Germany) after one year of observation.

Methods: Globally, the AHEAD study will enroll a total of >1000 severe to moderate HA patients. The observation period per subject will be ≥ 4 years. Study endpoints include long-term joint health outcomes, annualized (joint) bleeding rates (ABR/AJBR), factor consumption, health related quality of life and safety¹.

Results: The interim analysis of September 2014 includes 287 patients, of whom 162 completed the year 1 and 79 the year 2 visits. Median age at enrolment was 12 years (range 0–70) and 69% of patients had severe HA (FVIII<1%); 219 (81%) were on prophylaxis, 48 (18%) were OD and 3 (1%) on ITI treatment. Thirty-seven percentage of patients had target joints at screening. During the first year of observation 34 (27%) patients on prophylaxis did not experience any bleeding events (BE). For patients OD, most BEs were spontaneous (58%) while for patient on prophylaxis the BEs were equally traumatic or spontaneous. Most frequent bleeding sites were joints and muscles. Median ABRs were 1.2 (0–27) in the prophylaxis group and 11.9 (0–51) for OD; AJBRs were 1 (0–27) and 7.3 (0–36) in the two treatment groups. 49.5% of patients on prophylaxis and 20.8% of patients OD had an AJBR <1, while 66.7% of OD patients had an AJBR of >6. Median annualized total dose was 202,560 IU for prophylaxis and 35,346 IU for OD. Effectiveness of prophylaxis was excellent/good in 96% of cases. Effectiveness for treating an acute BE was excellent/good in 93% of cases for prophylaxis and 76% for OD.

Conclusion: Interim results of the AHEAD study show low ABR/AJBR for patients on prophylaxis with rAHF (ADVATE) in routine clinical practice, with good effectiveness rates.

Reference: ¹Oldenburg et al, EAHAD 2015.

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PO244-TUE

The ahead study: safety real world data after 1 year of observation, an analysis of the European cohort of patients

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Background: The AHEAD (ADVATE Haemophilia A outcome Database) study captures long-term outcome data in patients with haemophilia A (HA) receiving rAHF (ADVATE) under routine clinical practice and is a key source of real-world safety data.

Aims: We report interim safety results after one year of observation.

Methods: Globally the study will enroll a total of >1000 severe to moderate HA patients, receiving rAHF, regardless the number of previous exposure days (EDs) to FVIII concentrates. The observation period per subject will be ≥ 4 years. Study endpoints have been communicated elsewhere¹. Informed consent was obtained from all patients and the study was approved by the relevant ethics committees.

Results: The interim analysis of September 2014 includes 287 patients from European countries of whom 162 completed 1 year of observation. Median age at enrolment was 12 years (range 0–70) and 69% of patients had severe HA (FVIII<1%). During the first year there were 338 Adverse Events (AEs) in 82 patients. Only 4 AEs were assessed as treatment-related: one mild cutaneous allergic reaction with rhinitis and 3 clinically significant (CS) low-titre inhibitors (0.66–1.88 BU): 2 in minimally treated patients (MTPs) after 11 and 20 exposure days and 1 in a previously treated patient (PTP) with a history of an inhibitor at screening which became clinically significant during the study (0.66 BU). All patients had severe HA and all continued to receive rAHF. There were 15 serious AE (SAE) in 13 patients: Only the 3 low-titre inhibitors were related to rAHF. No deaths were reported, and no SAE was leading to study drug discontinuation. There was 1 life-threatening SAE (skull fracture with intracranial haemorrhage after accidental fall).

Conclusion: The AHEAD study reports real-world safety data, reassuring the good safety profile of rAHF (ADVATE) when used in clinical daily practice, both in previously treated, untreated or minimally treated (PTP/PUP/MTP) severe and moderate HA patients.

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PO245-TUE

A core set of outcome measurement tools in patients with hemophilia: a systematic review of the measurement properties

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Background: In patients with haemophilia the evaluation of outcomes in all three domains of the ICF model (1. body structures and function, 2. activities and 3. participation) is critical to determine appropriate management for individual patients in clinical care and to compare the effects of treatment strategies. A large number of tools are available. In order to improve quality of clinical management and clinical research, we aim to reach consensus on a selection of instruments. These measurement tools need to be valid, reliable and responsive to change. Therefore, a systematic evaluation of the measurement properties of potential candidate tools is needed.

Aims: We are in the process of performing a systematic review to assess the measurement properties of potential candidate outcome measurement instruments used in adult and pediatric patients with hemophilia. A secondary aim is to identify gaps in the current knowledge on measurement properties in order to direct further research.

Methods: A survey among comprehensive haemophilia treatment centres identified a set of outcome measurement tools which are included in the systematic review; 1. Petterson/IPSG/HEAD US scales, WFH/CPE/HJHS scores, 2. (Ped)HAL/FISH/COPM, 3. IPA, CAPE&PAC, Participation Scale and generic and disease specific HRQoL questionnaires. A systematic literature search to identify all available studies on these tools has been performed in Medline and Embase. The methodological quality of the studies is being critically appraised using the QUADAS2 and COSMIN checklists. Measurement properties include reliability (internal consistency, reliability, measurement error), validity (content validity, construct validity, criterion validity) and responsiveness. The quality of the outcome measurement tools will be appraised by the rating proposed by the Cochrane Back Review Group.

Results: The results and.

Conclusion: Conclusions of this International initiative will be submitted to the ISTH as soon as they are available.

Disclosure of Interest: None declared.

PO246-TUE

Clinical significance of type 1, type 2 T cells, TH17 and Treg in the development of FVIII inhibitor in pediatric hemophilia A patients

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Background: Higher incidence of inhibitory antibodies was produced related to replacement therapy with factor VIII (FVIII) in hemophilia A (HA) patients, which was the most high-risk complication in severe HA.

Aims: This study aims to investigate the immune regulatory role of Th17, Treg, Type 1 and type 2 T cells in the development of FVIII inhibitor in pediatric HA.

Methods: Total of 122 pediatric HA patients in single center of China were enrolled. The percentage of four type T cells was detected in peripheral blood mononuclear cells from HA patients by flow cytometric analysis.

Results: There were 36 cases (29.5%) of HA with positive FVIII inhibitor (higher titer 13/36, 36.1% and lower titer 23/36, 63.9%). Treg cells were higher in positive inhibitor of HA patients than that in the group of negative inhibitor HA ($7.17 \pm 1.47\%$ vs. $6.49 \pm 1.32\%$) ($P < 0.05$). Furthermore, compared with the lower titer of inhibitor of HA patients group, the higher percentage of Tc1 was observed in the higher titer of inhibitor group ($50.46 \pm 24.76\%$ vs. $32.23 \pm 16.38\%$) ($P < 0.05$). Also, similar tendency of Th1 as well as ratio of Th1/Th2 was found in the higher titer of inhibitor group, however did not achieve the significant difference (For Th1, $25.62 \pm 14.23\%$ vs. $16.87 \pm 11.50\%$, $P = 0.052$; for Th1/Th2, $28.01 \pm 31.58\%$ vs. $14.27 \pm 11.62\%$, $P = 0.067$, respectively).

Conclusion: Our findings suggest that higher Treg cells might be associated with the development of FVIII inhibitor in pediatric HA. Furthermore, Tc1 might be a risk factor to predict the severe status of inhibitor.

Disclosure of Interest: None declared.

PO247-TUE

Evaluate the quality of life in the elderly with haemophilia

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Background: Haemophilia had been effected on quality of life in the elderly patients.

Aims: The aim of this study was to evaluate the quality of life in the elderly with haemophilia.

Methods: The study was done for 6 months on the elderly was hemophilic. All the elderly with haemophilia who referred to Imam Khomeini hospital in haemophilia clinic were evaluated by a questionnaire (WHOQOL)-BREF questionnaire.

Results: of 30 patients were 6 women (20%) and 24 male (80) have been evaluated. the average age of the patients was 64.83 ± 5.9 years. physical health area average was 16.32 ± 3.42 , psychological area was 4.32 ± 16.74 , social environment area was 11.605 ± 5.775 and social communication area was 16.02 ± 3.18 . There was a Significant differences in difference areas based on type of haemophilia, gender, educational level, socio-economic status, medical insurance coverage.

Conclusion: In elderly people with haemophilia, there was a specific problems, such as inject able pharmaceuticals and the problems they experience, most of these patients after any detailed are traumatized, however, unfortunately, often the elderly patient experiencing disabilities resulting from haemophilia.

Disclosure of Interest: None declared.

PO248-TUE

Results of ankle prosthesis in patients with severe haemophilic arthropathy – follow up

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Background: Arthrodesis is predominantly used in cases of haemophilic arthropathy of the ankle joint. The prosthetic replacement surgery can offer a new therapeutic option for ankle arthropathy, and is a well established operation in patients with rheumatoid arthritis and in post traumatic arthritis. Few cases are published about ankle replacement in haemophilic arthropathy.

Aims: The aim was to evaluate the efficacy of ankle prosthesis in patients with severe haemophilic arthropathy.

Methods: Five patients with haemophilia A (severe: 4, mild: 1; 30–44 years), 1 female with von Willebrand disease type 3 (vWD) (45 years) and 1 patient with a severe deficiency of factor V showed an advanced state of joint destruction of the ankle evaluated by MRI/X-rays. The presence of severe pain, radiological joint damage (MRI score/Pettersson score) and a sufficient residual condition of mobility were the main indications for ankle replacement therapy. Surgical interventions were performed under FVIII or FVIII/VWF replacement therapy and the daily monitoring of the substitution.

Results: No complications (infection, intra-articular ankle bleeding) and side effects were documented in any patient. Typical lymphatic oedema was resolved after 6 months. Three years after ankle replacement, the patient with vWD required revision surgery due to a progressive decrease in mobility.

After follow-up of 2–8 years all prostheses were still in place and did not show any signs of loosening. Clinical scores showed a good ($n = 2$) to excellent ($n = 4$) result in the patients.

Conclusion: The ankle prosthetic replacement surgery represents a therapeutic option in patients with haemophilic arthropathy. Prospective studies are needed to confirm the efficacy of ankle replacement compared with that of ankle fusion.

Disclosure of Interest: None declared.

PO249-TUE

Immune tolerance induction in hemophilia A and B with inhibitor. single centre experience

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Background: Immune tolerance induction (ITI) successfully eradicates inhibitors in 60–80% and 30–50% of hemophilia A (HA) and hemophilia B (HB) patients with inhibitors, respectively.

Aims: To evaluate the ITI outcomes in 16 severe hemophilia patients with inhibitors diagnosed between the years 1994 and 2014 and treated in our Centre.

Methods: During the above mentioned period 15 HA patients received ITI: 14 with plasma derived Factor VIII concentrates containing von Willebrand Factor (FVIII/vWF) and 1 with recombinant FVIII (rFVIII). In HA a low dose (LD) regimen (FVIII $2 \times 50 \text{ IU kg}^{-1}$ 14 days followed by $1 \times 50 \text{ IU kg}^{-1}$ 14 days and then 50 IU kg^{-1} quod) and high dose (HD) protocol (FVIII $2 \times 100 \text{ IU kg}^{-1} \text{ day}^{-1}$) were employed. One HB patient with inhibitor received HD ITI ($2 \times 100 \text{ IU pdFIX}$) with initial concurrent immune modulation (dexamethasone, ivIgG, rituximab). Standard criteria for a complete success (CS) were used in terms of inhibitor negativity, factor recovery and T1/2.

Results: All patients had pre-ITI inhibitor titre $<10 \text{ BU mL}^{-1}$. The LD ITI was used in 9 HA pats (age 1–57 years; 6LR/3HR; median historical inhibitor titre 3.5 BU mL^{-1} ; range $2\text{--}17 \text{ BU mL}^{-1}$) with FVIII/vWF (8) and rFVIII (1). All (100%) achieved CS in a median of 12 (3–20) months. Inhibitor relapse in 58 years old patient was successfully treated with a second LD ITI combined with rituximab. HD ITI with FVIII/vWF was used in 6 high responders (1.5–16 years) with historical titre of 8 BU mL^{-1} ($7\text{--}500 \text{ BU mL}^{-1}$), 5/6 (83%) achieved CS after 12 months of ITI (range 8–36 months). In one patient the ITI is still ongoing (24 months). HB patient (6 years) with historical inhibitor titre of 75 BU mL^{-1} achieved a CS after 8 months ITI. Concurrent immune modulation successfully suppressed allergic reactions observed at the beginning of the ITI.

Conclusion: In hemophilia A we achieved in a total 93% success with either LD or HD ITI employing mostly FVIII/vWF concentrates. In hemophilia B immune suppression facilitated successfully to accomplish full course of ITI.

Disclosure of Interest: None declared.

PO250-TUE

PUPs with severe haemophilia A – a GCP study evaluating the immunogenicity, efficacy and safety of Human-cl rhFVIII

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Background: Human-cl rhFVIII is a human cell-line derived, B-domain deleted recombinant FVIII concentrate for intravenous use. Due to the absence of immunogenic epitopes, present in rFVIII concentrates from hamster cell lines, Human-cl rhFVIII is thought to be potentially less immunogenic. So far, 6 prospective GCP studies with Human-cl rhFVIII were conducted in almost 200 previously treated adults and children with severe haemophilia A (HA).

Aims: Since early 2013, a prospective, multinational, open-label, non-controlled clinical study is ongoing to assess the immunogenicity, efficacy and safety of Human-cl rhFVIII in previously untreated patients (PUPs).

Methods: Hundred evaluable patients with severe HA without previous exposure to any FVIII concentrate or FVIII containing products will be enrolled from 45 centers worldwide. Inhibitor testing according to modified Bethesda method will be performed pre-treatment, every 3–4 exposure days (ED 1–20) and every 10–12 EDs (ED 21–100) but at least every three months after start of treatment. Efficacy and safety of Human-cl rhFVIII is assessed during on-demand or prophylactic therapy, breakthrough bleeds treatment, and in surgical prophylaxis.

Pharmaco-economic aspects and predictive factors for the development of inhibitors will be evaluated. Several optional sub-studies to identify predictive markers of inhibitor development in order to accurately identify individuals at risk of inhibitor formation in PUPs have been initiated.

Results: By the end of January 2015, 40 sites worldwide had been initiated in 15 countries. A total of 53 PUPs had been screened at 21 sites in 11 countries, and 43 of them had started treatment with *Human-cl rhFVIII*. Safety data are continuously reviewed by an IDMC. No safety issues were detected so far. First preliminary results will be presented during summer 2015.

Conclusion: A global GCP-study in PUPs is ongoing in order to investigate whether the reduced immunogenic profile of *Human-cl rhFVIII* will translate into a lower inhibitor incidence.

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PO251-TUE

Population pharmacokinetics in hemophilia A: towards individualization of perioperative replacement therapy

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Background: Pharmacokinetic (PK)-guided dosing has been proven efficacious in prophylactic treatment of haemophilia A patients. However, few data are available on the possible benefits of perioperative PK guided FVIII dosing.

Aims: To facilitate Bayesian adaptive dosing we conducted a retrospective study to construct a perioperative population PK model for (moderate) severe hemophilia A patients.

Methods: Hemophilia A patients (FVIII levels <0.05 IU ml⁻¹) were included, undergoing elective, low or medium risk surgery between 2000 and 2012 from five Academic Hemophilia Treatment Centers in the Netherlands. Data was collected on FVIII treatment (dose, brand of concentrate), patient and surgical characteristics. Population PK modeling was performed using nonlinear mixed-effects modeling (NONMEM). The study was not subject to the Medical Research Involving Human Subjects Act and approved by the Medical Ethics Committee.

Results: Population PK parameters were estimated in 75 adults (140 surgeries; median age: 48 years, median weight: 80 kg) and 44 children (58 surgeries; median age: 4.3 years, median weight: 18.5 kg). PK profiles were best described by a two-compartment model. Typical values for clearance (CL), intercompartmental clearance, central (V1) and peripheral volume were 0.16 L h⁻¹/68 kg, 0.17 L h⁻¹/68 kg, 2.82 L/68 kg and 1.88 L/68 kg. Interpatient variability in CL and V1 was 37% and 27%. CL decreased with age ($P < 0.01$); increased in case of blood group O (27%, $P < 0.01$) and was dependent on the severity of

surgical procedure (7%, $P < 0.01$) and the presence of a bleeding complication (13%, $P < 0.01$).

Conclusion: A population model was developed describing the perioperative PK of FVIII concentrates, allowing the individualization of perioperative FVIII dosing by Bayesian analysis. Therapeutic drug monitoring of perioperative FVIII may prevent current underdosing and overdosing, thereby leading to decreased FVIII consumption and lowering of treatment costs without loss of quality of care.

Disclosure of Interest: None declared.

PO252-TUE

Prenatal diagnosis for haemophilia: umbilical cord blood sample used

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Background: Haemophilia A (HA) and B (HB) are the two most common X-linked inherited bleeding disorders. Prenatal diagnosis is an alternative method to avoid patient was born. However, some family members couldn't perform the gene diagnosis owing to the proband's samples absent for any reasons. The pregnant women also need to do the prenatal diagnosis for haemophilia.

Aims: To set up a prenatal diagnosis method for haemophilia family female members who are not suitable for gene diagnosis.

Methods: Anticoagulant umbilical cord blood samples were collected at 21–23 weeks gestation. The global coagulation tests and all factor activities were assessed on IL coagulation analyser. Totally 48 samples have been detected, including 35 samples for haemophilia A diagnosis and 13 for haemophilia B diagnosis.

Results: 12 haemophilia A and 5 haemophilia B fetus have been diagnosis according the factor activity results from umbilical cord blood samples. The mean value for fibrinogen is 0.87 g L⁻¹. The mean values for factor II, factor V, factor VII, factor X, factor VIII, factor IX, factor XI and factor XII are 29.26%, 78.81%, 25.91%, 25.10%, 37.74%, 11.80, 20.87%, 20.87%, separately.

Conclusion: The main risk for prenatal diagnosis for haemophilia using umbilical cord blood sample is the maternal blood contamination. In our study, we use the global coagulation tests combined the factor activity detection to identify whether the umbilical sample collection is successful. The results demonstrate that it's a good method for prenatal diagnosis for haemophilia if one family is not suitable for gene diagnosis for any reason.

Disclosure of Interest: None declared.

PO253-TUE

Bleeding affects health-related quality of life (HRQoL) in people with haemophilia patients (PWH) – even a single bleed mattersvon Mackensen S¹, Eichler H², Holstein K³, Khair K⁴ and Schramm W⁵¹Institute of Medical Psychology, University Medical Centre Hamburg-Eppendorf, Hamburg, Georgia; ²Institute of Clinical Haemostaseology and Transfusion Medicine, Saarland University Medical Center, Homburg/Saar; ³II. Medical Clinic and Policlinic, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany; ⁴Great Ormond Street Hospital for Children NHS Trust, London, UK; ⁵Department of Haemostaseology and Transfusion Medicine, Ludwig-Maximilians University Munich, Munich, Germany**Background:** Animal studies and clinical data have shown that each avoided bleed matters in the prevention of joint damage. Poor evidence is available on its impact on health-related quality of life (HRQoL).**Aims:** Evaluation of HRQoL in people with haemophilia (PWH) with 0 bleeds in comparison with patients with bleeding events.**Methods:** Data from different national and international studies in adult and paediatric PWH were analysed to verify the hypothesis that a single bleed already impacts their HRQoL. In these studies HRQoL was assessed with generic instruments (adults: SF-36, EQ-5D; children: KINDL) and/or haemophilia-specific instruments (adults: Haem-A-QoL, MedTap; children: Haemo-QoL). The time frame of bleeding rate varied across the studies from 'no of bleeds and joint bleeds in the previous 6 months' to 'annual rate of bleeds and joint bleeds'.**Results:** The European ESCHQoL Study showed that PWH with 0 joint bleeds in the previous 6 months had a significant better HRQoL. Adults ($n = 900$) reported better values in all domains of the SF-36 and the MedTap questionnaire; similarly, adolescents ($n = 138$) reported a significant better HRQoL in most domains of the Haemo-QoL. In a Study from the UK ($n = 84$) children with 0 joint bleeds in the past 6 months had a significant better Total Score of the Haemo-QoL. In a German study in adult patients ($n = 56$) PWH with 0 joint bleeds in the previous year reported significant better HRQoL in the domains 'physical health', 'sport', 'dealing' and 'treatment' in the Haem-A-QoL. Another German study showed that adults ($n = 58$) with 0 joint bleeds in the previous year had better HRQoL in the domains 'physical functioning', 'bodily pain', 'general health' and in the Physical Component Score of the SF-36.**Conclusion:** These findings demonstrate that PWH with 0 bleeds have better HRQoL. Not only joint health, but also HRQoL is affected by even a single bleed.**Disclosure of Interest:** S. von Mackensen Grant/Research Support from: Wyeth UK, European Commission, H. Eichler Grant/Research Support from: Baxter, K. Holstein Grant/Research Support from: CSL Behring Germany, K. Khair Grant/Research Support from: Wyeth UK, W. Schramm Grant/Research Support from: European Commission.

PO254-TUE

Intracranial hemorrhage and management in hereditary bleeding disorders: the experience of Cukurova University, Adana, TurkeySasmaz I¹, Antmen B², Karagun BS², Yilmaz DM³, Leblebisatan G¹ and Kilinc Y¹¹Pediatric Hematology, Cukurova University; ²Pediatric Hematology, Acibadem Hospital; ³Neurosurgery, Cukurova University, Adana, Turkey**Background:** Intracranial hemorrhage (ICH) is a life threatening complication of hereditary bleeding disorders in childhood resulting in high rates of mortality and disabling sequelae.**Aims:** In this study, we evaluated our patients with intracranial hemorrhage and compared with literature.**Methods:** From 1995 to 2014, 27 patients with intracranial hemorrhage were diagnosed and evaluated in Çukurova University Hemophilia Center. ICH episodes, the findings of physical examination, CT scan or MRI and treatment strategies including surgical interventions were reviewed retrospectively.**Results:** We evaluated 28 episodes of ICH from 27 patients with hereditary congenital factor deficiencies (CFD). Age range was from 9 days to 12 years. There were 18 patients with hemophilia A, 2 patients with hemophilia B, 2 patients with factor VII deficiency, 4 patient with factor X deficiency, 1 patient with von Willebrand disease. Two patients with factor VII deficiency, 2 patients with factor X deficiency and 1 patient with von Willebrand disease were female. Except one patient with factor X deficiency, all patients had one bleeding episode. Three patients had a high titer inhibitor against factor VIII. The most important factor was trauma. A history of recent trauma was documented in 15 patients. Intracerebral and subdural hematoma were more frequently seen. The most frequent symptoms were seizure and headache. The diagnosis of hemophilia was established in 5 patients after intracranial hemorrhage who referred to our center with ICH. In 11 patients, hematoma was evacuated. Three hemophilia patients died due to ICH and 5 patients presented late sequelae.**Conclusion:** Intracranial hemorrhage is the most serious complication in childhood, especially for hereditary bleeding disorders. Urgent establishing diagnosis and treatment with prompt doses of factors to initially maintain a normal concentration of circulating factor is mandatory. Despite the prompt treatment, death and late sequelae can be seen in this patients group.**Disclosure of Interest:** None declared.

PO255-TUE

Exploring cost effectiveness of various prophylactic treatment strategies for severe haemophilia using a computer simulation modelFischer K^{1,2} and Janssen M²¹Van Creveldkliniek; ²Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands**Background:** Severe hemophilia requires life-long treatment with expensive clotting factor concentrates; modeling techniques are used to compare effects of different treatment strategies over decades.**Aims:** To explore strategies for switching between prophylactic and on demand treatment according to different prophylactic dose levels using an updated computer model.**Methods:** The previously developed computer simulation model was updated with data from a formal expert elicitation process. Additional information included life expectancy, treatment of minor bleeding episodes, and prophylactic dose needed to control bleeding according to the patients' onset of joint bleeding. The model was used to simulate individual patients' life time joint bleeds, radiological outcome (Pettersson score, max 78 points) and concentrate use according to different treatment strategies. Based on the cost effectiveness (maximum % of patients with a Pettersson score <15 at a minimum cost) different strategies for switching between prophylactic and on demand treatment according to different prophylactic dose levels of 1500 to 3500 IU kg⁻¹ year⁻¹ were explored.**Results:** According to the model, a life-long prophylactic regimen with a dose of 2000 IU kg⁻¹ year⁻¹ was most cost effective, maintaining a Pettersson score of <15 points at the end of life in 62% of patients. Including switching reduced this proportion slightly to 60%. Optimum switching criteria were similar across prophylactic dose levels: patients could switch to on demand treatment if they had suffered ≤ 1 joint bleed/in the last two years on an average prophylactic dose; prophylactic

laxis should be restarted if patients suffered a maximum of 8 joint bleeds in one year, 6 year⁻¹ in two years, or 4 year⁻¹ in three years while treated on demand.

Conclusion: The present model suggests that optimum criteria for switching between prophylaxis and on demand treatment are independent of prophylactic dose and that prophylaxis at 2000 IU kg⁻¹ year⁻¹ is most cost-effective.

Disclosure of Interest: None declared.

PO256-TUE

Ultrasound evaluation of haemophilic joints by haemophilia physicians: a reliability study

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Background: The assessment of joint health is a key aspect of monitoring treatment efficacy in haemophilia. Detecting early synovial and cartilage changes to guide treatment strategy is a challenge; MRI is cumbersome and expensive, but ultrasound (US) is promising. Recently, the HEAD-US technique and score was developed to facilitate bedside US evaluation by non-radiologists.

Aims: To assess the validity and inter-operator reliability of the HEAD-US score performed by haemophilia physicians.

Methods: On two consecutive days, 5 haemophilia treaters and one reference operator independently examined a total of 42 joints: 2 joints in each of 21 patients using identical US machines. The HEAD US score evaluates synovium (0–2 points), cartilage (0–4 points) and bone (0–2 points), resulting in a total score ranging from 0 (no changes) to 8 points (severe changes). Spearman correlation with the reference score, internal consistency (Cronbachs alpha), inter-observer agreement (intraclass correlation coefficient (ICC)) and limits of agreement according to Bland and Altman were calculated.

Results: Operators were haemophilia physicians, all had limited training of 2.5 days (in two sessions) and a short calibration session prior to the study. Median age was 29.9 years (range 16–47). Fifteen Ankles, 12 knees, and 15 elbows were scanned, the median score was 3.5 (IQR 1–6) with 21% no abnormalities and 2% maximum scores. Correlation with the reference rater was good (range 0.66–0.89), with good internal consistency (Alpha 0.94). Inter-observer agreement was high with an ICC of 0.72 (95% CI 0.62–0.82). At joint level, the limits of agreement were 3.3 points.

Conclusion: Ultrasound evaluation of haemophilic joints with the HEAD US score can be performed by haemophilia physicians after only limited training. This technique and scoring system may take a central role in the continuous evaluation of joint health and tailoring-prophylaxis. Agreement between operators was already good and is expected to increase with further experience.

Disclosure of Interest: K. Fischer Consultant for: Member of the Pfizer Ultrasound Steering Committee, J. Oldenburg Consultant for: Member of the Pfizer Ultrasound Steering Committee, J. Astermark Consultant for: Member of the Pfizer Ultrasound Steering Committee, G. Dolan Consultant for: Member of the Pfizer Ultrasound Steering Committee, M. Morfini Consultant for: Member of the Pfizer Ultrasound Steering Committee, N. Marquardt Consultant for: Pfizer, C. Martinoli Consultant for: Member of the Pfizer Ultrasound Steering Committee.

PO257-TUE

Dosing of rVIIa-FP in clinical studies in hemophilia with inhibitors and factor VII deficiency

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Background: Current difficulties in treatment of patients with congenital Hemophilia with Inhibitors (CHwI) and patients with FVII Deficiency (FVIIDef) are caused by the short plasma half-life of rFVIIa of 2.5–3 h, necessitating several injections per day to treat most bleeds and making a treatment schedule for use in prophylaxis a challenge. rVIIa-FP is a new fusion protein of rFVIIa and recombinant albumin with a significantly prolonged half-life of 8.5 h, which was developed to address these problems.

Aims: Define dosing and schedules to be tested in the clinical development program of rVIIa-FP in the indications CHwI and FVIIDef.

Methods: Peak plasma activities of rVIIa-FP were obtained in a single dose PK study in healthy human volunteers after injection of rVIIa-FP at 5 different dose levels. These were compared to rFVIIa peak plasma activity after injection of 90 µg kg⁻¹ and the dose equivalent of rVIIa-FP to rFVIIa was calculated.

Results: The dose equivalent of rVIIa-FP to 90 µg kg⁻¹ of rVIIa was calculated at 573 µg kg⁻¹. Upcoming clinical trials in CHwI will test the efficacy and safety of 0.75 and 1.5 mg kg⁻¹ rVIIa-FP to control and prevent bleeding; in upcoming clinical trials in FVIIDef, 0.10–0.4 mg kg⁻¹ will be utilized.

Conclusion: PK studies allow estimating equivalent dosing of rVIIa-FP to established doses of rFVIIa across different indications. The significantly prolonged half-life of 8.5 h may translate to a more sustained control of hemostasis after injection of a single dose.

Disclosure of Interest: B. Britta Laros-van Gorkom: None declared, P. Holme: None declared, W. van Heerde: None declared, J. Mahlangu: None declared, F. Dato' Abd Karim: None declared, P. Chowdary: None declared, D. Bensen Kennedy Employee of: CSL Behring, A. Veldman Employee of: CSL Behring, R. Clementi Employee of: CSL Behring, C. Nergier: None declared.

PO258-TUE

Efficacy and safety of rVIII-singlechain in surgical prophylaxis

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Background: rVIII-SingleChain, a novel recombinant Factor VIII, has been designed as a B-domain truncated construct with a covalent bond between the heavy and light chains with a higher binding affinity to von Willebrand Factor. rVIII-SingleChain has a lower clearance, longer half-life and larger area under the curve compared to octocog alfa.

Aims: This sub-study investigated the safety and efficacy of rVIII-SingleChain when used in the perioperative setting for prevention and bleeding control in patients with severe Hemophilia A undergoing major surgery.

Methods: The study was approved by Ethics committees of all sites and conducted according to GCP and the Declaration of Helsinki. Dosing was guided by the current WFH recommendations. Dosing was according to individual patient PK and was given by bolus or continuous infusion.

Results: 13 patient underwent 16 major surgeries which included extraction of wisdom teeth, abdominal hernia repair, elbow replacement, ankle arthroplasty, knee replacement (5), cholecystectomy, lengthening of the achilles tendon combined with straighten up of the right toes, circumcision (3), open reduction Internal fixation right ankle fracture and hardware removal right ankle. Eight procedures were covered by bolus infusion., and 8 by continuous infusion of rVIII-SingleChain. Investigators rated the efficacy of rVIII-SingleChain to provide hemostasis during surgery as excellent (defined as hemostasis not clinically significant different from normal) in all cases but one in which it was rated as good (defined as hemostasis normal or mildly abnormal in terms of quantity and/or quality eg, slight oozing). After the procedure, patients returned to routine treatment after a median of 9 days. No related AEs or SAEs were observed during the peri-surgical period.

Conclusion: rVIII-SingleChain is efficacious and safe when used for peri-surgical prophylaxis and bleeding control during a wide range of surgical procedures when dosed following the WFH recommendations.

Disclosure of Interest: C. Djambas Khayat: None declared, J. Mahlangu: None declared, C. Leisinger: None declared, S. P'ng: None declared, E. Santagostino: None declared, F. Lopez Fernandez: None declared, D. Bensen Kennedy Employee of: CSL Behring, A. Veldman Employee of: CSL Behring, C. Regina Employee of: CSL Behring, I. Pabinger: None declared.

PO259-TUE

Inhibitors in Hemophilia (HA): experience of a single center in the last 22 years

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Background: Inhibitor development is the most serious treatment complication in HA.

Aims: To investigate the incidence of inhibitor development in our HA population in the last 22 years.

Methods: All patients were diagnosed between 1992 and 2014.

Results: 153 patients received a diagnosis of HA (54 severe, 14 moderate and 85 mild). Seventy-three/153 (48%) have been exclusively treated with recombinant FVIII (rFVIII) at least once in their life: 11/73 (15%) developed an inhibitor [7 high responder (HR), 3 low responder (LR), 1 transient]. Fourteen/153 (9%) have been exclusively treated with plasma-derived FVIII (pdFVIII) at least once in their life: 3/14 (21%) developed a HR inhibitor. Seven/153 (4.5%) have been treated either with pdFVIII or rFVIII: 1/7 (14%) developed a LR inhibitor. Globally, 15/94 (16%) treated patients, all severe, developed an inhibitor: 10 HR, 4 LR, 1 transient. As regard as FVIII gene mutation, we observed Intron 22 inversion in 5 patients, nonsense mutations in 4, deletions in 2, Intron 1 inversion in 2, a frameshift mutation in 1. One patient is still under study. Three/15 patients presented a family inhibitor history. Prior to inhibitor development, 5/15 patients underwent an intensive treatment. Eight/10 patients with HR inhibitor, underwent ITI: 7 obtained a CR, 1 is ongoing. Four patients with LR and 1 patient with transient inhibitor, underwent prophylaxis regimen with FVIII concentrate: at last follow-up just one of them is still positive for a LR inhibitor.

Conclusion: Inhibitor incidence in our population is 16%. The incidence of inhibitor in patients exclusively treated with either rFVIII or pdFVIII is 15% and 21% respectively. Mild or moderate hemophilia A patients did not develop inhibitors. No missense mutations were detected in our inhibitor patients. Eleven/15 patients developed an inhibitor after <50 EDs (five patients <20 EDs).

Disclosure of Interest: None declared.

PO260-TUE

Systematic literature review of economics analyses comparing APCC and rFVIIa across on-demand, prophylaxis and surgery

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Background: Prior systematic reviews on economic comparison of activated prothrombin complex (aPCC) & recombinant factor VIIa (rFVIIa) reviewed studies related to on-demand (OD) bleeds in hemophilia inhibitor patients. The bypassing agents (BA) are also used for prophylaxis & surgery. Given the economic burden of BA it is crucial to have valid cost-effectiveness estimates for BA across all three indications.

Aims: To conduct a systematic review of economic studies comparing BA (i.e. aPCC and rFVIIa) across OD, prophylaxis & surgery indications.

Methods: Biosis preview, Embase & Medline were searched (1993–2014) for published economic studies comparing BAs. Information on

sponsorship, study design, model assumptions and results were collected and evaluated for each study.

Results: A total of 17 economic studies were included in the review (OD = 16; prophylaxis = 2; surgery = 1). The majority of the studies were sponsored by aPCC ($n = 4$) or rFVIIa ($n = 10$) manufacturer. All studies adopted a third-party payer perspective. A majority ($n = 11$) of OD economic models used similar decision tree & assessed cost/bleeding episode. One OD study assessed differential cost saving with use of bypass therapy assay testing. The assumptions on BA efficacy and dosing drove study conclusions. A cost minimization analysis that assumed equal efficacy favors aPCC in OD. The surgery economic model assessed cost savings across BA dosage regimen in orthopedic surgery patients. The prophylaxis studies were conducted from a retrospective perspective and studied prophylaxis cost in conjunction with OD costs. All but one study favored their sponsor's product. Although studies were few, limited information available in the prophylaxis and surgery indications tend to be in favor of aPCC treatment.

Conclusion: Although clinical superiority hasn't been scientifically established for either BA, across indications and studies, clinical parameter assumptions drove the cost-effectiveness conclusions for each BA.

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PO261-TUE

Addressing the gap in treatment adherence: development of the hemophilia joint visualizer tool

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Background: Prophylaxis with replacement clotting factor prevents bleeding episodes and arthropathy, thus preserving joint function and improving quality of life (QOL) for persons with hemophilia.

Aims: To evaluate adherence patterns and develop an educational tool to visualize long-term impact of non-adherence on the joints.

Methods: Patients on prophylaxis were evaluated via 3-week diary and survey in a quantitative segmentation study ($N = 181$). Those with low adherence (≥ 2 missed scheduled infusions in 3 months; $n = 51$) were assessed for treatment behaviors. A web-based hemophilia joint visualizer (HJV) tool was developed to visualize the long-term impact of short-term choices on joint health.

Results: Non-adherence was reported in 40% of prophylaxis patients, with an average 5 infusions missed in 3 months. Risk for discontinuing prophylaxis was 15%. Non-adherence was associated with disease condition, patient attitude, infusion routine and relationship with healthcare provider (HCP). Among non-adherent patients, 38% unintentionally missed infusions; 45% intentionally skipped infusions due to self-management of schedules or belief that infusing is related to activity level. Intentional skippers believed that bleeding frequency was not impacted by strict schedule adherence. The HJV tool was developed as an educational tool to aid HCPs in communicating the value of prophylaxis on long-term joint health. The tool personalizes a patient's experience based on prophylaxis time, age and adherence, and presents a visual impact assessment for those who are non-adherent or who switch to on-demand therapy. An individualized joint profile summary is provided, which facilitates discussion with HCPs about impact of projected joint health on activities.

Conclusion: Non-adherence to prophylaxis can lead to deteriorating joint function and QOL in persons with hemophilia. The HJV tool may be valuable for improving adherence through education about long-term impact of non-adherence on joint health.

Disclosure of Interest: H. Takedani Consultant for: Bayer, Speaker Bureau of: Bayer, Baxter, Novo Nordisk, Pfizer, Kakestuken, L. Solimeno Consultant for: Bayer, Baxter, Novo Nordisk, K. Saxena Employee of: Bayer HealthCare Pharmaceuticals, P. Mathew Employee of: Bayer HealthCare Pharmaceuticals.

PO262-TUE

rVIII-singlechain pharmacokinetics in adults, adolescents and children

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Background: rVIII-SingleChain, a novel recombinant Factor VIII (rFVIII), has been designed as a B-domain truncated construct with a covalent bond between the heavy and light chain, aiming for a higher binding affinity to von Willebrand Factor (vWF). Since vWF is one of the main determinants of FVIII plasma survival, this increased binding affinity might translate into improved pharmacokinetics of rVIII-SingleChain.

Aims: Two studies investigated the pharmacokinetics (PK) of rVIII-SingleChain, one in children (<12 years, ongoing) and the other in adolescents and adults (12–<18 and 18–65 years) with severe Hemophilia A.

Methods: The studies were approved by the relevant Ethics committee and national authorities and conducted according to GCP and the Declaration of Helsinki. PK samples of rVIII-SingleChain were collected prior to infusion (pre-dose) and at 0.5, 1, 4, 8, 10, 24, 32, 48, 72 and 96 h post-infusion in adults and adolescents and prior to infusion (pre-dose) 1, 4 to 6, 10, 24, and 48 h post-infusion in children. Plasma FVIII activity was measured in the blood samples using the chromogenic substrate assay.

Results: The preliminary PK results are as following. In adults and adolescents ($n = 35$), the mean PK parameters after rFVIII-SingleChain dosing were 14.1 h for $t_{1/2}$, 2.89 mL h⁻¹ kg⁻¹ for CL, 20.2 h for MRT and 1931 IU*h dL⁻¹ for AUC_{inf}. In the younger children (0–<6 years, $n = 6$), they were 8.7 h for $t_{1/2}$, 5.26 mL h⁻¹ kg⁻¹ for CL, 11.4 h for MRT and 987 IU*h dL⁻¹ for AUC_{inf}. In the older children (6–<12 years, $n = 6$), they were 10.0 h for $t_{1/2}$, 4.71 mL h⁻¹ kg⁻¹ for CL, 12.7 h for MRT and 1169 IU*h dL⁻¹ for AUC_{inf}.

Conclusion: The increased binding of rVIII-SingleChain to vWF translates into favorable pharmacokinetics without the need for glycopegylation or fusion to antibody fragments. Expectedly, mean $t_{1/2}$ was shorter and mean CL greater in children compared to adults and adolescents.

Disclosure of Interest: J. Mahlangu: None declared, L. Lepatan: None declared, K. Vilchevska: None declared, J. Oldenburg: None declared, O. Stasyshyn: None declared, K. Fischer: None declared, G. Iosava: None declared, C. Djambas Khayat: None declared, M. Simpson: None declared, M. Wang: None declared, D. Bensen Kennedy

Employee of: CSL Behring, N. France Employee of: CSL Behring, K. St. Ledger Employee of: CSL Behring, T. Limsakun Employee of: CSL Behring, I. Pabinger: None declared.

Heparin-induced thrombocytopenia (HIT) I

PO263-TUE

Argatroban in the management of heparin-induced thrombocytopenia type II: an open-label, multicenter clinical trial in France

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Background: Argatroban is a synthetic thrombin inhibitor that offers potential benefit to critically ill patients with Heparin-induced thrombocytopenia Type II (HIT).

Aims: To collect data in France in patients with HIT who were eligible for parenteral antithrombotic therapy with argatroban.

Methods: 20 patients (mean age 72 ± 10) were enrolled in this open-label, multicenter clinical study and treated in 7 centers over a period of 15 months. Argatroban treatment was monitored using aPTT and anti IIa activity.

Results: The diagnosis of HIT was confirmed in 16 subjects by an independent scientific committee. Fourteen patients (70%) were in an intensive care unit (ICU) during the course of the study. Patients were treated with argatroban for a mean duration of 8.5 ± 6.1 days. The mean starting dose of argatroban was $0.77 \pm 0.45 \mu\text{g kg}^{-1} \text{min}^{-1}$ and remained stable all over the period of treatment (mean dose: $0.78 \pm 0.44 \mu\text{g kg}^{-1} \text{min}^{-1}$). Platelet recovery was rapid ($147 \pm 89 \text{ g L}^{-1}$ on day 3, $215 \pm 102 \text{ g L}^{-1}$ on last day of treatment). aPTT and anti-IIa activity assays were used to monitor the dose of argatroban. The mean baseline aPTT value was $45.0 \pm 9.8 \text{ s}$ and increased to $78.2 \pm 35.8 \text{ s}$ 2 h after initiating argatroban. At this time mean argatroban concentration was 0.28 ± 0.34 and $0.38 \pm 0.31 \mu\text{g mL}^{-1}$ using ECT and TT measurements respectively. New and/or extended thromboses were reported in 25% of patients and major bleedings were documented in 15%. Six patients died due to their underlying medical condition. No amputations were reported.

Conclusion: In ICU setting, argatroban can be safely used for the treatment of HIT in elderly and frail patients who present at the same time a high thrombotic and hemorrhagic risk. Because aPTT baseline is more often prolonged in ICU patients, anti IIa activity can be useful to monitor the treatment. However, it has to be taken into account when monitoring argatroban that the 2 assays measuring anti IIa activity do not give superimposable results.

Disclosure of Interest: B. Tardy Consultant for: Mitsubishi Tanabe Pharma Corporation, LFB, P. Nguyen: None declared, J.-C. Thiranos: None declared, P.-E. Morange Consultant for: Leo pharma, BMS-Pfizer, Boehringer Ingelheim, Bayer healthcare, C. Biron Andréani: None declared, Y. Gruel: None declared, J. Morel: None declared, A. Wynckel: None declared, L. Grunebaum: None declared, J. Villacorta-Torres: None declared, S. Grosjean: None declared, E. De Maistre Consultant for: LFB.

PO264-TUE

Case report: dabigatran use in a postoperative orthopedic patient with heparin induced thrombocytopenia

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Background: Heparin induced thrombocytopenia (HIT) is an adverse effect of heparin therapy. Approved treatment is parenteral direct thrombin inhibitors, which require frequent monitoring, can cause serious bleeding and are expensive. Dabigatran, an oral direct thrombin inhibitor, can be an alternative therapy.

Aims: To present a case in which dabigatran was used off-label for the treatment of HIT, after approval by local ethical committee.

Methods: A 67 years old woman was submitted to a total knee replacement and began enoxaparin 40 mg day^{-1} 12 h after surgery. Ten days later she presented with severe headaches, without trauma. A computed tomography (CT) scan identified an intracranial hemorrhage due to a bleeding transformation of a cerebral venous thrombosis. Laboratory tests results were: hemoglobin 9.3 g dL^{-1} , platelet count (plt) $58 \times 10^9 \text{ L}^{-1}$ (preoperative plt: $208 \times 10^9 \text{ L}^{-1}$), CrCl 65 mL min^{-1} , normal coagulation tests, D-Dimers $52 \mu\text{g mL}^{-1}$ ($N < 0.5$). Several thrombotic events were diagnosed: right cavernous sinus thrombosis, left internal jugular vein thrombosis, right renal arterial thrombosis, deep venous thrombosis of right leg and pulmonary embolism.

Results: HIT was suspected (pretest clinical score: 8) and heparin/PF4 antibodies detection tests performed were positive. After repeating CT scan, argatroban was begun on day 2 (dose $2 \text{ mg kg}^{-1} \text{min}^{-1}$) with periodic titration. On day 19, plt remained low ($81 \times 10^9 \text{ L}^{-1}$). It was decided to switch to dabigatran 150 mg bid , according to an investigator study. Plt count increased to $147 \times 10^9 \text{ L}^{-1}$ at day 4 of dabigatran and remained normal. Patient was discharged on day 34 with clinical recovery.

Conclusion: This case illustrates the severity of thrombotic events of HIT. Argatroban is the approved therapy but had no success in this case. Dabigatran can be an attractive option due to its oral administration, no monitoring needed and low bleeding risk. It has been used in few cases of HIT. Further information is needed to determine its efficacy and safety.

Disclosure of Interest: None declared.

PO265-TUE

Rivaroxaban for treatment of heparin induced thrombocytopenia

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Background: HIT is a pro-thrombotic and potentially lethal disorder caused by antibodies against platelet factor 4 (PF4)/heparin complexes. Apart from increased morbidity and mortality, it also leads to increased length of hospital stay and significant health care costs.

Aims: To describe a case of HIT successfully treated with Rivaroxaban.

Methods: An 84-year-old gentleman presented with new onset altered mental status. On physical exam, he had Cheyne-Stokes breathing and was responsive to painful stimuli. CT head did not show any bleed, hence he received tissue plasminogen activator (t-PA). His initial com-

plete blood count showed WBC of 7.97 k mm^{-3} , hemoglobin 13.8 g dL^{-1} and Platelets $203 \text{ k } \mu\text{L}^{-1}$. He was started on DVT prophylaxis 24 h post t-PA with Heparin 5000 units subcutaneous Q8 h. On day 4, platelets dropped to 72 k mm^{-3} . On Day 5, patient developed a spontaneous left brachial vein thrombosis. His 4T pretest probability score for HIT was 7 (high).

Results: His heparin was discontinued and he was started on Rivaroxaban 15 mg orally twice a day for 21 days followed by 20 mg Qdaily. His heparin induced platelet antibody was elevated at 0.415. His serotonin release assay was positive at 20% confirming the diagnosis of HIT. His platelet count improved on Rivaroxaban to 229 k mm^{-3} by Day 13. Three months post HIT; his platelets continue to be stable.

Conclusion: Rivaroxaban is an oral direct Factor-Xa inhibitor shown to be effective in prevention and treatment of venous thromboembolism (VTE); and in stroke prevention in atrial fibrillation. Its role in HIT has not been studied or established. Two independent *in vitro* studies have shown Rivaroxaban has no effect on the interaction of PF4 or anti-PF4/heparin Abs with platelets. Our case demonstrates its utility in successful management of HIT. Although a larger sample size is required to establish its efficacy and risk profile in HIT patients, *in vitro* studies and theoretical explanation supports its use as a plausible alternative to direct thrombin inhibitors.

Disclosure of Interest: None declared.

PO266-TUE

The incidence of heparin-induced thrombocytopenia in critically ill medical patients

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Background: Thrombocytopenia is very common in critically ill medical patients and heparin-induced thrombocytopenia (HIT) is often one of the major concerns. Our previously reported series revealed that these patients comprised the majority of the documented HIT cases, but they often showed falsely low pre-test probability scores due to the concomitant illnesses. However, the data of HIT in this group is lacking.

Aims: This study aimed to determine the incidences of positive HIT antibody (HITAb) and HIT development in medical critical care units.

Methods: We performed a prospective cohort of critically ill medical patients, who received any forms of heparin between November 2013 and December 2014 at King Chulalongkorn Memorial Hospital. Blood samples from all patients were collected for a platelet factor 4/heparin-particle gel immunoassay (PaGIA) at day 7 after heparin exposure. Cases yielding PaGIA+ were subject to perform the heparin-induced platelet aggregation (HPA) test to confirm HIT diagnosis.

Results: There were 105 patients enrolled. The mean age was 65.1 ± 18 years. Fifty five (52.4%) were female. Ninety four (89.5%) had a fall in platelets $>30\%$. Of 18 (17.1%) with PaGIA+, there was only 1 (0.9%) confirmed HIT by HPA. Cases with PaGIA+ were found in 11/86 (12.8%), 6/17 (35.3%) and 1/2 (50%) in low, intermediate and high probability of the 4Ts score, respectively. The PaGIA+ results were found more frequently in cases using unfractionated heparin than in cases using low molecular weight heparin (16/82, 19.5% vs. 2/23, 8.7% $P = 0.35$). However, there were no variables significantly associated with a positive HITAb screening in multivariate analysis. The documented HIT case received enoxaparin due to atrial fibrillation with unstable hemodynamics and yielded the 4Ts score of 6 and the HEP score of 11.

Conclusion: The incidences of positive HITAb screening and confirmed HIT in critically ill medical patients in our cohort were 17.1% and 0.9%, respectively. The patient enrolment of this study is ongoing.

Disclosure of Interest: None declared.

PO267-TUE

Evaluating efficacy, safety and compliance of argatroban dosing nomogram at Canadian teaching hospital

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Background: Heparin-induced thrombocytopenia (HIT) is an immune-mediated adverse drug reaction. Direct thrombin inhibitors, like argatroban, are used to treat HIT. An argatroban dosing nomogram is used at University Health Network.

Aims: Evaluate 3 key areas of nomogram: efficacy, safety and percent compliance

Methods: Patients who received argatroban from July 2012 to February 2013 were retrospectively identified using dispensing data.

Efficacy outcome was defined as time to therapeutic activated partial thromboplastin time (aPTT).

Safety outcome was defined as blood transfusion following drop in hemoglobin $\geq 10 \text{ g L}^{-1}$.

Percent compliance was defined as number of infusion rate-change decisions that followed the nomogram, divided by the total for each patient.

Electronic patient records were used to obtain data such as infusion rate changes, aPTTs, blood transfusions administered etc.

Results: of 17 identified patients, enzyme immunoassays were performed for 16 patients; 11 were positive. Additionally, 6 out of 9 patients were confirmed HIT positive with serotonin release assays.

For efficacy and compliance analysis 14 patients were included based on having >1 argatroban rate change.

Eight patients reached therapeutic, as predefined by the nomogram, aPTT of 75–95. Median time to first therapeutic aPTT was 2.5 days (Interquartile range (IQR) 2–3.25).

Five patients experienced bleeds during argatroban therapy with a median hemoglobin drop of 14.5 g L^{-1} (IQR 10.75–78). Median aPTT at time of bleeds was 83 (IQR 52.5–92).

Preliminary data showed no thrombotic events despite subtherapeutic aPTTs.

Compliance was calculated for each patient with a median compliance of 100% (IQR 77.68–100).

Conclusion: Many patients receiving >2 days of therapy had subtherapeutic aPTTs as predefined by the nomogram regardless of appropriate infusion rate increases suggesting that the desired range of aPTT is too high.

No relationship between aPTT and bleeds was apparent.

While the nomogram was easy and safe to follow, aPTT targets need to be re-evaluated.

Disclosure of Interest: None declared.

PO268-TUE

Rapid functional assay for heparin-induced thrombocytopenia using a practical flow cytometry approach

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Background: Heparin-induced thrombocytopenia (HIT) is characterized by thrombocytopenia and thrombotic complications secondary to the formation of IgG antibodies against heparin-platelet-factor 4 (PF4) complexes that bind to platelet FC receptors. This is followed by events including platelet activation, aggregation and rapid elimination of platelets, associated with morbidity and mortality. Common laboratory testing, required for the confirmation of HIT, is based on

immune detection of antibodies to the PF4/heparin complex. However, these assays have low specificity, compared to the platelet functional assays. The 'gold standard' functional test for detecting platelet-activating antibodies is the [^{14}C] serotonin-release assay (^{14}C -SRA) (Sheridan D. et al, *Blood* 1986;67:27), is not commonly performed as it includes the use of a radiolabeled C^{14} and requires expertise to obtain reliable results.

Aims: To overcome limitations of the current assays, we modified a functional flow-cytometry assay (FCA) which exhibits high sensitivity and specificity (Tomer A. et al, *Am J Hematol* 1999;61:53). This assay, similar to the ^{14}C -SRA, determines the capacity of the patient's serum to activate platelets in the presence of heparin, using a fluorescent probe.

Methods: Consecutive samples from 457 patients clinically suspected for HIT were tested. The FCA assay was compared with the standard ID-H/PF4 PaGIA assay (DiaMed, Switzerland) with two added dilutions to assess specificity (Nellen V. et al, *Haematologica* 2012;97:89).

Results: Seventeen percent (77/457) of all samples were positive by PaGIA, compared to 3.3% (15/457) positive by FCA. The number of PaGIA positive samples was reduced to 7% (31/457) by 1:16 dilution and to 4% (17/457) by 1:32 dilution. All FCA positive samples were PaGIA positive (RS 88%). All PaGIA negative samples were FCA negative (RS 100%).

Conclusion: The results suggest that the functional FCA is a rapid sensitive and highly specific test for the reliable diagnosis of HIT.

Disclosure of Interest: None declared.

PO269-TUE

Heparin-induced thrombocytopenia and calibrated thrombin generation assay

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Background: Heparin-induced thrombocytopenia (HIT) is a severe adverse drug reaction of heparin treatment. Platelets activated by HIT sera generate procoagulant platelet-derived microparticles, leading to thrombin generation. Tardy et al. demonstrated with thrombin generation assay the central paradox of HIT, i.e. a procoagulant effect in presence of heparin.

Aims: The primary objective of this study was to optimize our protocol of thrombin generation assay. The second objective was to test HIT suspected patients to determine if thrombinography might have a place in the diagnosis of HIT.

Methods: Thrombin generation was assessed by the calibrated automated thrombogram. Different parameters were evaluated for the development of this assay. Three inducers were tested. Two low concentrations of unfractionated heparin (UFH) were evaluated: 0.2 and 1 IU mL⁻¹. To ensure heparin dependence of platelet activation, a condition with a high concentration of UFH at 100 IU mL⁻¹ was investigated. Protamine was used to inhibit anticoagulant heparin effect in the experiment. Patients suspected of HIT with a positive ($n = 10$) and a negative diagnosis ($n = 37$) were tested with the optimized protocol.

Results: PRP-Reagent was selected as the inducer reagent. Donor PRP and patient PPP were incubated with saline or UFH at 0.2 IU mL⁻¹. Among HIT-positive patients, the addition of UFH enhanced the thrombin generation, showing 'a HIT thrombogram profile'. Among the HIT-negative patients, 9 patients presented an unexpected HIT thrombogram profile. This discrepancy is not yet addressed. Besides, studies on the activity and amount of platelet-derived microparticles are ongoing.

Conclusion: HIT thrombogram profile demonstrates the procoagulant effect in presence of heparin for HIT positive patients. In this pilot

study, a high number of false positive was obtained suggesting that thrombin generation assay may not be a useful diagnosis assay for HIT.

Disclosure of Interest: None declared.

Immune thrombocytopenia (ITP) II

PO270-TUE

The role of cellular immunity in children with idiopathic thrombocytopenic purpura

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Background: To evaluate the role of cellular immunity in idiopathic thrombocytopenic purpura (ITP).

Aims: To indicate the role of Th1 and Th2 lymphocytes and cytokine profiles released from Th1 lymphocytes and Th2 lymphocytes in patients with acute ITP in the pathogenesis of acute and chronic ITP.

Methods: The study included 23 patients with acute ITP, ten patients with chronic ITP, and 14 healthy children as the control. IFN γ , IL-4 and TGF- β 1 levels were evaluated in patients with acute ITP at the diagnosis, and in responder and non-responder patients after IVIG and high dose methylprednisolone (HDMPZ) therapy and in patients with chronic ITP.

Results: IFN γ and IFN γ /IL-4 values of the patients with acute ITP at the time of diagnosis were not statistically higher than the controls ($P = 0.47$ and $P = 0.06$, respectively) and TGF- β 1 was lower compared to the controls ($P = 0.001$). IFN γ , TGF- β 1, and IFN γ /IL-4 values of the patients with chronic ITP were lower than the controls ($P > 0.02$, $P = 0.37$, and $P = 0.06$, respectively). IFN γ and IFN γ /IL-4 values in patients with chronic ITP were lower than the patients with acute ITP ($P = 0.01$), IFN γ and IFN γ /IL-4 values in patients with acute ITP who responded or did not respond to therapy were higher than the controls ($P = 0.01$), and there was no statistically significant difference between TGF- β 1 levels ($P = 0.10$). IFN γ /IL-4 values of patients with acute ITP who were unresponsive to the therapy were higher compared to the values of responders ($P = 0.08$), and TGF- β 1 and IL-4 levels were lower compared to responders ($P = 0.10$, $P = 0.39$, respectively).

Conclusion: The findings revealed that the pathogenesis of acute ITP is associated with cellular immunity such as T lymphocytes, monocytes and macrophages; however, cellular immunity cannot be directly implicated in chronic ITP, and other factors might have played a role in its pathogenesis.

Disclosure of Interest: None declared.

PO271-TUE

Diagnosis of venous thromboembolism during eltrombopag therapy for immune thrombocytopenia and successful replacement with romiplostim

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Background: There has been growing evidence concerning the development of venous thromboembolism (VTE) during eltrombopag therapy in patients with refractory immune thrombocytopenic purpura (ITP). However, the feasibility of switching between thrombomimetic agents post VTE development has not been fully explored to date.

Methods: We present clinical data from 2 female patients, (75 and 68 years old) diagnosed with refractory ITP (after failing at least steroids and rituximab), who developed VTE during eltrombopag therapy and were then successfully transferred to romiplostim.

Results: VTE events – pulmonary embolism (PE) and sinus vein thrombosis (SVT), were diagnosed at 30 and 58 days respectively post initiation of eltrombopag. Platelet (PLT) counts at thrombomimetic initiation were $4000 \mu\text{L}^{-1}$ (PE) and $10,000 \mu\text{L}^{-1}$ (SVT) and at the time of VTE development were $<50,000 \mu\text{L}^{-1}$. Neither of these patients were suitable for a splenectomy due to various co-morbidities. Upon diagnosis of VTE, eltrombopag was stopped and enoxaparin initiated for one patient. A superior vena cava filter was inserted for the patient with PE, due to an inability to administer anticoagulation in the presence of a very low PLT count ($<10,000 \mu\text{L}^{-1}$). Romiplostim was introduced at 15 and 100 days post VTE. PLT counts at that time were 13,000 and $15,000 \mu\text{L}^{-1}$ respectively and increased within 14 days. To date, one patient has received romiplostim for 26 months (in combination with enoxaparin) and the other for 2 months with no new embolic events.

Conclusion: These case reports support the feasibility of replacing eltrombopag therapy with romiplostim in conjunction with anticoagulation, for patients diagnosed with VTE during treatment with eltrombopag for refractory ITP.

Disclosure of Interest: None declared.

PO272-TUE

Re-evaluation of need for bone marrow examination in patients with isolated thrombocytopenia treated with steroids as first line therapy

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Background: Diagnosis of Immune thrombocytopenia (ITP) is based on clinical suspicion and normal peripheral smear except for thrombocytopenia. Bone marrow examination is carried out to rule out leukemia, MDS or aplastic anemia. However, in most cases, clinical diagnosis is not altered after the bone marrow reports. Hence, this present study was carried out to evaluate the justification for bone marrow examination in the setting of isolated thrombocytopenia.

Aims: To study the need for bone marrow examination in isolated thrombocytopenia.

Methods: All patients presenting with isolated thrombocytopenia and suspected diagnosis of ITP, between October 2011 and April 2013, were included. Data was collected from bone marrow reports and outpatient records.

Results: A total of 353 cases were found. Three hundred and nineteen cases were having features of typical ITP and the rest 34 cases had mild organomegaly or lymphadenopathy. There were 14 (3.9%) patients more than 60 years of age, 143 (40.51%) in the age group 19–40 years and the remaining <18 years. The most common symptom was petechiae (75.92%), epistaxis (32.86%), menorrhagia in 30.9% females. Twenty-four patients also had fever and 29 (8.2%) had weakness. One hundred and eighty-seven patients had newly diagnosed ITP, 82 had persistent ITP and 84 had chronic ITP.

All cases were managed as ITP, and none of them have developed features to suggest an alternate diagnosis on followup (median follow up 48 months; range 12–144 months). Bone marrow examination in all cases revealed normal hematopoietic elements and prominence of megakaryocytes including juvenile forms with no novel diagnosis in any patient.

Conclusion: Routine use of bone marrow examination in the diagnostic workup of isolated thrombocytopenia is unnecessary in our center even if steroids are planned as a first line therapy. However, a detailed

history, thorough examination with complete hemogram and peripheral smear examination are necessary to avoid a bone marrow examination. A bone marrow examination is to be advised in the presence of any atypical feature

Disclosure of Interest: None declared.

PO273-TUE

Glycoprotein IB α clustering induces macrophage-mediated platelet clearance in the liver

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Background: Many immune thrombocytopenia (ITP) patients, particularly patients with anti-glycoprotein (GP) Ib-IX autoantibodies, do not respond to the conventional treatments such as splenectomy. However, the underlying mechanism remains unclear.

Aims: To investigate the mechanism of anti-GPIb-IX autoantibodies induced platelet clearance.

Methods: Antibodies-induced platelet aggregation was detected by an optical aggregometer. GPIb α clustering was measured by Fluorescence Resonance Energy Transfer. Antibodies-induced platelets depletion and rescue experiments were performed in guinea pigs and cynomolgus macaques. Platelet distribution after antibody-mediated depletion was detected by *in vivo* Imaging System (IVIS), immunohistochemistry and immunofluorescence microscopy.

Results: We found that anti-GPIb α N-terminus antibody AN51, but not other anti-GPIb α antibodies (AK2, HIP1, VM16d, or WM23), induced integrin $\alpha_{IIb}\beta_3$ -dependent platelet aggregation. FRET result showed that AN51 induced obvious GPIb α clustering but not VM16d. After intravenous injection, AN51 dose-dependently induced thrombocytopenia in guinea pigs, and the platelets were mainly removed by macrophages in the liver. N-acetyl-D-glucosamine (GlcNAc), previously shown to inhibit $\alpha_{IIb}\beta_3$ -mediated phagocytosis of refrigerated platelets, showed dose-dependent inhibition of AN51-induced platelet destruction. Furthermore, AN51 but not VM16d, induced rapid platelet clearance in the liver of cynomolgus macaques. Five of twenty-two chronic ITP patients had anti-GPIb α autoantibodies, and the autoantibodies from four of the five patients competed with AN51 for binding to platelets.

Conclusion: GPIb α clustering induced by anti-GPIb α N-terminus antibody causes integrin $\alpha_{IIb}\beta_3$ -dependent platelet aggregation, phagocytosis, and rapid platelet clearance in the liver. Our findings reveal a novel Fc-independent mechanism underlying the pathogenesis of ITP, and suggest new therapeutic strategies for ITP patients with anti-GPIb α autoantibodies.

Disclosure of Interest: None declared.

PO274-TUE

Extended platelet parameters as a means to differentiate immune thrombocytopenia from hypoproliferative thrombocytopenias

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Background: Immune thrombocytopenia [ITP] remains a diagnosis of exclusion. The Siemens ADVIA 120 has the capacity to calculate the mean platelet [PLT] component [MPC], a measure of platelet density, and the mean platelet mass [MPM], neither of which are currently employed in clinical decision making.

Aims: To determine if the MPC and MPM are significantly higher in ITP (reflecting increased platelet granules) than in hypoproliferative thrombocytopenia (HT).

Methods: Institutional review board approval was obtained. A prospective study was initiated in September 2013. This trial is registered at ClinicalTrials.gov. Patients at Mount Sinai Roosevelt Hospital with ITP, as defined by the ASH 2011 practice guidelines, and HT's (delineated in Results section) were included. Patients with human immunodeficiency virus, hepatitis C, cirrhosis, pregnancy, and disseminated intravascular coagulation were excluded. Enrollment of 20 patients in each arm was planned. Lavender tri-potassium EDTA tubes were filled and analyzed on the Siemens ADVIA 120 within a period not exceeding 2 h from their collection. MPC and MPM values between ITP and HT patients were compared utilizing the student's t-test.

Results: Twenty patients with ITP and 20 patients with HT (4 aplastic anemia, 8 chemotherapy-induced thrombocytopenia, 3 myelodysplasia, 1 acute myelogenous leukemia, 1 hairy cell leukemia, 1 multiple myeloma, and 2 drug-induced thrombocytopenia (valproic acid, imatinib) were enrolled. Baseline characteristics were similar. Median age, M:F ratio, and mean PLT count were 54 years, 0.67:1, and $62,500 \mu\text{L}^{-1}$ (ITP) and 60.5 years, 1:1, and $53,900 \mu\text{L}^{-1}$ (HT), $P > 0.05$. MPC (g dL^{-1} , mean $\pm$ SD) was 26.4 ± 1.89 (ITP) and 24.2 ± 1.89 (HT), $P < 0.01$. MPM (pg, mean $\pm$ SD) was 2.46 ± 0.474 (ITP) and 1.95 ± 0.210 (HT), $P < 0.01$.

Conclusion: MPC and particularly the MPM are significantly higher in ITP than in HT. Extended platelet parameters hold promise for distinguishing ITP from other thrombocytopenias.

Disclosure of Interest: None declared.

PO275-TUE

Treatment of atraumatic splenic rupture in an adolescent with Systemic Lupus Erythematosus (SLE) and autoimmune cytopenias: a case report

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Background: Atraumatic rupture of spleen is a rare but life-threatening complication of systemic lupus erythematosus (SLE). Management of atraumatic splenic rupture in a patient with SLE who has active autoimmune cytopenias is challenging.

Aims: To report the hemostatic management in a patient with SLE who had active autoimmune cytopenias and atraumatic rupture of spleen and required an urgent abdominal surgery.

Methods: We reported a patient with SLE who had atraumatic rupture of spleen with autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia (ITP).

Results: A 17-year-old Thai girl with SLE had a new onset of gum bleeding and pallor. Complete blood count showed low hemoglobin (Hb) 5.9 g dL^{-1} and platelet count (plt) $16,000 \text{ mm}^{-3}$ with positive Coombs' tests and increased reticulocyte count (RC). She was treated with pulse methylprednisolone 1 g for 3 days followed by oral prednisolone, however, the condition did not improve. On the 4th day of admission, she developed acute abdominal pain with guarding and more pallor. Her Hb and plt decreased to 3.9 g dL^{-1} and 7000 mm^{-3} , respectively. Serum amylase and lipase were normal. Computed tomography (CT) of abdomen revealed a large hematoma in gastrohepatic region and ruptured pseudoaneurysm of left gastric artery. She received pulse methylprednisolone 1 g for 3 days, intravenous immunoglobulin (IVIG) 40 g for 2 days, tranexamic acid, most-compatible red blood cell concentrate 260 ml and platelet concentrate (PC) 8 units (U) before undergoing splenectomy. During surgery, she bled 300 ml and received PC 6 U, cryoprecipitate 8 U and recombinant activated factor 3600 μg . She was admitted for 4 weeks and discharged uneventfully.

Conclusion: Atraumatic splenic rupture should be considered as a cause of acute abdomen in patients with SLE developed progressive

abdominal pain. Hemostatic control in patients with active autoimmune cytopenias during the major surgery could be achieved by multimodality treatment.

Disclosure of Interest: None declared.

PO276-TUE

CD70 molecule involved in the dendritic cells dysfunction in patients with immune thrombocytopenia

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder. Dysfunction of dendritic cells (DCs) is considered to be involved in the pathogenesis of ITP. CD70 is transiently and stimulation-dependently expressed on DCs. There had been reports showing that CD70 was over-expressed in lymphocytes of ITP patients and anti-CD70 antibodies could rectify the over-apoptosis of platelets caused by auto-antibodies in ITP patients.

Aims: This study aimed to investigate the function of CD70 in DCs of ITP patients.

Methods: Mature DCs were induced from the isolated CD14⁺ leukocytes from PBMCs of ITP patients and normal controls. CD4⁺ T cells, CD4⁺CD25⁻ T cells and CD19⁺B cells from normal controls were also isolated. Induced DCs stimulated by anti-CD70 or not were then sent to coculture at 1:5 and 1:10 ratio with normal CD4⁺ T cells and B cells. Then the proliferation and the cytokine secretion were tested. DCs were also cocultured at 1:2 and 1:5 ratio with CD4⁺CD25⁻ T cells. Five days later Treg cells differentiation were detected and the cells proliferation and the cytokine secretion related to Tregs were also determined.

Results: DCs from ITP patients expressed lower CD1a, suggesting there might be maturation disorder existing. In the coculture with CD4⁺ T cells, both DCs from ITP patients and normal controls stimulated by anti-CD70 could promote the proliferation of T cells and the Th1-related cytokine secretion at any ratio. And the promotion was more significant in stimulated DCs from ITP patients. for Tregs differentiation, DCs from normal controls could induce larger percent of Tregs from CD4⁺CD25⁻ T cells than ITP patients. Meanwhile, the DCs stimulated by anti-CD70 induced less Tregs than DCs without stimulation at any ratio in ITP patients, and anti-CD70 also decreased the cells proliferation of Tregs differentiation.

Conclusion: The presence of anti-CD70 promoted the hyper-function in DCs of ITP patients, presented as the enhancing of the CD4⁺ T cells proliferation and the weakening of Tregs differentiation.

Disclosure of Interest: None declared.

PO277-TUE

Megakaryocytes and macrophage -lymphocyte interactions *in vitro* in patients with immune thrombocytopenia

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Background: Though immune thrombocytopenia (ITP) is well studied disease, antibodies against platelets aren't detected in 20–40% to confirm immune conflict. In some cases of thrombocytopenia (TP)

because of impaired production of thrombopoietin (Tpo) Tpo receptors are offered. Therefore to reveal immune conflict is important. Investigation of thrombopoiesis (Tpoiesis) is also a challenging problem in idiopathic and symptomatic TP.

Aims: Aim of our study was to reveal immune conflict by high amount of MLRs and observe Tpoiesis *in vitro* in 15 patients with TP.

Methods: We used leukocyte culture methods worked out by us that support cells to maintain their proliferation activity and function (Shvelidze, Saralidze Atlas of Hematology, 2013). Macrophage (Mf)-lymphocyte (L) interaction was studied according to MLRs formation in 3-day blood leukocyte cultures. Tpoiesis was observed in 5–9 days bone marrow (BM) leukocyte cultures.

Results: High amount of MLRs –60–78% (percentage of Mf contacted with L) was revealed in 12 cases of TP (9-ITP, 3-TP in patients with SLE), pointing to the immune genesis of TP. In donors this index is 35–39%. In 3 patients with liver cirrhosis and TP MLRs was 20–25%, pointing to nonimmune genesis of TP. In ITP patients after treatment with steroids amount of MLRs was decreased to 25–30% showing effectiveness of therapy. In patients with ITP normal megakaryocytes (MG) containing mainly 6–7 fragmented nucleus with normal or increased platelet release were observed, while in patients with liver cirrhosis and TP MG contained 3–5 fragmented nucleus and Tpoiesis was decreased. In acute megakaryoblastic leukemia amount of MG was increased revealing their misshaped forms with 1–3 nuclear fragments and producing immature platelets.

Conclusion: MLRs formation *in vitro* can be successfully used for estimation of immune conflict and effectiveness of therapy while BM culture is essential to observe Tpoiesis in TP patients and reveal abnormalities of hematopoiesis and precise diagnosis.

Disclosure of Interest: None declared.

PO278-TUE

The involvement of Neuropilin-1 in primary immune thrombocytopenia

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Background: In the immune system, Neuropilin-1 (Nrp-1) is a molecule that plays an important role in establishing the immunological synapse between dendritic cells (DCs) and T cells, which is essential in the priming of resting T cells and contributes to the development of peripheral tolerance.

Aims: In this study, we investigated the relationship between the expression of Nrp-1 and its ligands Semaphorins-3A (Sema3A), Transforming Growth Factor- β 1 (TGF- β 1) as well as Th1/Th2, which probably contributes to the pathogenesis of primary ITP in Chinese population.

Methods: 21 naïve ITP patients, 18 remission ITP patients and 23 ethnically matched controls were examined in this study. Flow cytometry was used to detect the expression of Nrp-1 on CD4+CD25+CD127 high Treg cells and Elisa kits were used to detect the level of Sema3A, TGF- β 1, INF- γ , IL-4 in plasma. Statistical analysis was performed using the SPSS19.0.

Results: Nrp-1 expression was evidently lower in naïve ITP patients ($0.12 \pm 0.07\%$) than controls ($0.45 \pm 0.11\%$) ($P < 0.05$), while remission ITP patients ($0.37 \pm 0.18\%$) have a similar level with controls ($P > 0.05$). The plasma Sema3A level in naïve ITP patients was remarkably higher than controls ($8.10 \pm 1.34 \text{ ng mL}^{-1}$ vs. $2.92 \pm 0.9 \text{ ng mL}^{-1}$, $P < 0.05$). However, the plasma Sema3A level in remission ITP patients did not decrease to a similar level with controls ($7.26 \pm 2.96 \text{ ng mL}^{-1}$ vs. $2.92 \pm 0.9 \text{ ng mL}^{-1}$, $P < 0.05$). The difference of plasma TGF- β 1 level between naïve ITP patients and controls was significant ($P < 0.05$). The plasma IL-4 level in these three groups

did not show significant difference ($P > 0.05$). The plasma INF- γ level in naïve ITP patients notably increased when compared with controls ($16.91 \pm 4.25 \text{ pg mL}^{-1}$ vs. $7.77 \pm 3.33 \text{ pg mL}^{-1}$, $P < 0.05$), therefore, the balance of Th1/Th2 was broke (1.21 ± 0.25 vs. 0.50 ± 0.22).

Conclusion: In this study, we demonstrated the relationship between Nrp-1 and its ligands as well as the balance of Th1/Th2. Obviously, our data showed that Nrp-1 played an important role in the pathogenesis of primary ITP.

Disclosure of Interest: None declared.

PO279-TUE

Successful treatment of refractory ITP with cyclosporine A, two case reports

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Background: Refractory immune thrombocytopenia is a serious clinical problem. There are no clearly successful treatment recommendations available for management of patients, who failed splenectomy and TPO-RAs. In symptomatic patients one of the other immunosuppressive approaches may be indicated, including cyclosporine A. Experiences with CyA in ITP therapy are limited.

Aims: Retrospective analysis of two interesting cases.

Methods: Case reports.

Results: We report two cases of patients with refractory ITP in whom pre-SE multiple immunosuppressive therapy including CyA was not effective. Treatment response was not achieved with SE either. As the first patient exhausted treatment options and was at a high risk for bleeding, an attempt was made to restart immunosuppressive therapy with CyA. Surprisingly, this treatment was effective. This experience was analogically applied in the other patient in a similar situation; once again, it was successful. Both patients achieved complete remission of ITP and the response continued after CyA was withdrawn. Treatment with CyA was well tolerated in both cases. Detailed data are presented on the poster.

Conclusion: The presented cases have confirmed the potential therapeutic effect of CyA in refractory post-SE disease. More interestingly, the immunosuppressive drug was not effective prior to SE. No reliable explanation for this clinical effect exists. It is likely that there is an interference with autoimmune mechanisms in ITP and these are modified after SE.

Disclosure of Interest: None declared.

PO280-TUE

Efficacy and prognosis of short term and very low-dose IVIG therapy ($200 \text{ mg kg}^{-1} \text{ day}^{-1}$) for newly diagnosed acute immune thrombocytopenic purpura in children

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Background: High-dose intravenous immunoglobulin G (IVIG) is the first therapy for childhood acute immune thrombocytopenic purpura (aITP). Individual low-dose method has been applied in the Department of Pediatrics, Kyungpook National University Hospital since

1984. We have reduced the daily dose from 400 to 200 mg kg⁻¹ daily until platelet count (PLT) over 50,000 µL⁻¹ since 2000.

Aims: This study evaluated the efficacy of short term and very low dose IVIG (VLD-IVIG) therapy according to individual clinical response.

Methods: Totally 46 childhood ITP Patients who's PLT <20,000 µL⁻¹ were evaluated from January 2007 to December 2013. We treated them with VLD-IVIG (200 mg kg⁻¹ day⁻¹ for 1–5 days) initially until rising PLT over 50,000 µL⁻¹ and then any patients without the response were treated additional IVIG up to 2 g kg⁻¹ totally.

Results: The mean age of 22 male and 24 female ITP was 26 months. The mean PLT was 8300 µL⁻¹ at the time of diagnosis. Thirty nine patients responded with VLD-IVIG only. Four patients needed only one day, 11 patients 2 days, 9 patients 3 days, 10 patients 4 days, and 5 patients 5 days of IVIG (200 mg kg⁻¹) respectively. Six of 7 patients who did not raise PLT above 50,000 with VLD-IVIG had response after full dose (total 2 g kg⁻¹). Only 1 patient did not respond with IVIG and needed corticosteroids and 1 patient recurred within 2 weeks after therapy.

The mean numbers of IVIG injection to 50,000 µL⁻¹ was 3 times (621 mg kg⁻¹ of dose). The mean PLT after therapy was 108,692 µL⁻¹. The mean duration to recover PLT more than 150,000 µL⁻¹ was 2 months. No patient was diagnosed to chronic ITP. The mean follow-up duration was 24 months and mean PLT was 336,410 µL⁻¹ at last follow-up date.

Conclusion: The short term VLD-IVIG therapy according to individual clinical response is effective and is recommended for acute ITP to reduce admission days and cost of high dose IVIG therapy.

Disclosure of Interest: None declared.

Inflammation II

PO281-TUE

A synthetic peptide, corresponding to the noncanonical PAR1- tethered ligand, mimics the APC-induced stimulation of wound healing *in vitro*

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Background: Activated protein C (APC) is a physiological anticoagulant with strong anti-inflammatory and cytoprotective activities, which appear to be protective in a number of disorders including chronic wound healing. The anti-inflammatory properties of APC are mediated via EPCR and PARs. Different from thrombin, APC cleaves PAR1 at the canonical cleavage site R41/S42, as well as at an alternative site R46/N47, releasing non-canonical tethered ligand, that activates PAR1. We assume that the cytoprotective effects of APC on the cells involved in wound healing can be simulated by the synthetic peptides – analogues of tethered ligand triggering PAR1.

Aims: The goal of this study was to investigate the effect of the synthesized peptide (AP) – analogue of the tethered ligand of PAR1, released by APC, on the proliferation of human keratinocytes and wound healing *in vitro*.

Methods: Quantitative assessment of EPCR and PAR1 expression in the culture of human keratinocytes was performed using flow cytometry using specific primary antibodies to PAR1 and to EPCR. MTT assay was used to quantitate the effect of test agents on cell growth and viability. Scratch test was used as an *in vitro* model of wound healing. The peptide NPNDKYEPF-NH₂ (AP9) was synthesized in Russian cardiology research and production complex.

Results: It was shown that a large part of the keratinocytes population (59.5 ± 11.8%) co-express PAR1 and EPCR. AP9 (0.1–10 µM), like

to APC (0.01–100 nM), induced cells proliferation in dose-dependent manner, maximum was at concentration 10 µM AP9 and 10 nM APC. AP9, as well as APC, accelerates wound healing *in vitro*. The width of the 1 nM APC and 10 µM AP9 treated wounds was significantly decreased and amounted to 388.9 ± 51.9 µm and 546.2 ± 112.6 µm respectively, the size of the untreated wounds was 888.9 ± 16.7 µm. Using specific antibodies it was shown that PAR1 and EPCR are involved in proliferative activity of agonists.

Conclusion: The results suggest the possibility of using a peptide AP9 to stimulate tissue repair.

Disclosure of Interest: None declared.

PO282-TUE

Differential *in vivo* activation of monocyte subsets during experimental endotoxemia in humans

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Background: Human monocytes are a heterogeneous cell population that can be divided into a classical (CM, CD14⁺CD16⁻), a non-classical (NCM, CD14⁺CD16⁺), and an intermediate subset (IM, CD14⁺CD16⁺). Monocytes are key cells in the response to sepsis.

Aims: A human endotoxemia model was used to identify monocyte subset activation under septic conditions.

Methods: Healthy volunteers (*n* = 12) were injected with a bolus infusion of LPS (2 ng kg⁻¹) and blood samples were obtained before LPS injection and at 2 h, 6 h and 24 h after injection. Whole blood samples were stained for CD14, CD16 and CD11b and were analysed with a BD FACS Canto II. Absolute cell numbers were determined using 123 counting beads and a novel *in situ* mRNA hybridization approach to detect IL6 and IL8 specific mRNA at the single-cell level by flow cytometry was applied.

Results: The analysis of cell counts showed a drop in monocyte levels after 2 h of LPS treatment. After 6 h, CM recovered to their initial cell number whereas IM and NCM remained reduced. After 24 h, monocyte subsets were skewed towards IM, which showed a 572% increase (*P* < 0.001). In addition, IM showed the strongest upregulation of CD11b after 2 h compared to CM and NCM (*P* < 0.05, *P* < 0.005). After 6 h and 24 h CD11b returned to baseline values albeit IM still displayed the highest baseline expression. Furthermore, IL6 and IL8 mRNA levels were enhanced after 6 h in IM to 180% (*P* < 0.05) and 240% (*P* < 0.05) respectively and NCM to 225% (*P* < 0.05) and 232% (*P* < 0.05) respectively, whereas CM response was weak to 119% (*P* < 0.05) and 142% (*P* < 0.05) respectively. After 24 h, IM and NCM mRNA levels for IL6 and IL8 mRNA returned back to the baseline expression levels.

Conclusion: These results show that monocyte subsets are activated differently during endotoxemia *in vivo*. Especially CD16 positive cells react strongly to LPS treatment resulting in higher CD11b, IL6 and IL8 levels compared to CD16 negative cells.

Disclosure of Interest: None declared.

PO283-TUE

Regulation of IL-10 and High Mobility Group Box 1 (HMGB1) expression in human monocytes by platelets and neutrophils

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Background: Development of atherosclerosis occurs ‘silently’ over several decades where the pro- as well as anti-inflammatory mediators play a central role. The cytokine IL-10 is a major anti-inflammatory cytokine whereas high mobility group box 1 (HMGB1) protein is secreted from activated monocytes/macrophages as a mediator of inflammation, and has been reported to be associated with atherosclerotic plaque composition and burden.

Aims: To explore the role of activated platelets and neutrophils in the production and secretion of IL-10 and HMGB1 in LPS-stimulated monocytes.

Methods: Monocytes and neutrophils were isolated from freshly drawn blood on Polymorphprep and re-suspended in plasma (Fragmin). Isolated cells were immediately recombined and subjected to stimulation by LPS (5 ng mL⁻¹) alone, LPS + PMA, or LPS + Ca-ionophore (A23187) for 2 h at 37 °C in a rotating incubator. After centrifugation of the samples, plasma was frozen until quantification of IL-10 and HMGB1 in ELISA kits.

Results: Platelets, as reported earlier, enhanced LPS-induced IL-10 in monocytes by 109% in our study whereas neutrophils reduced the enhancement by platelets, particularly when PMA was present. PMA or A23187 in addition to LPS, amplified the platelet effect of IL-10 production 4–11 fold. PMA had very little effect on IL-10 production in monocytes of whole blood in the absence of LPS, but increased HMGB1 levels in plasma by 144% compared to 64% by LPS alone and 304% by PMA and LPS in combination. In contrast to the production of IL-10, platelets had no effect whereas neutrophils increased LPS-induced HMGB1 by 92% and 196% in LPS + PMA stimulated monocytes. Platelets reduced this effect of neutrophils in LPS + PMA stimulated monocytes by 37%.

Conclusion: Activated platelets exert a strong amplification of LPS-induced IL-10 in monocytes whereas neutrophils inhibit this effect. HMGB1 production in LPS-stimulated monocytes is not affected by platelets but significantly upregulated by activated neutrophils.

Disclosure of Interest: None declared.

PO284-TUE

Effect of lipopolysaccharide and various cytokines on protein C inhibitor production by HepG2 cells

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Background: Protein C inhibitor (PCI), a member of SERPIN family, is expressed in various human tissues, including liver and kidney. PCI has procoagulant properties to inhibit anticoagulant serine protease, activated protein C (APC) and thrombin-thrombomodulin (TM) complex. In humans it is well known that patients suffering from sepsis

also have severe thrombotic tendency. However, the detailed mechanism is still unknown.

Aims: In the present study, to elucidate the mechanism of the thrombotic tendency observed in sepsis patients, we examined the effect of lipopolysaccharide (LPS) and various cytokines on the production of PCI in HepG2 cells.

Methods: The hepatoma cell line, HepG2 cells, were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum, 100 U mL⁻¹ penicillin and 100 µg mL⁻¹ streptomycin at 37 °C under 5% CO₂ atmosphere. Confluent HepG2 cells were treated with LPS, or various cytokines for 24 h, and then culture supernatants were collected by centrifugation at 15,000 rpm for 10 min. The concentration of PCI in culture supernatants of HepG2 cells was determined by ELISA specific for human PCI.

Results: LPS dose-dependently increased PCI production in HepG2 cells, whereas IFN-γ, IL-1β, and IL-6 decreased it. In addition, TNF-α had no effect on PCI production in HepG2 cells.

Conclusion: These results suggest that increase of plasma PCI induced by LPS is one of the causes of the thrombotic tendency observed in patients suffering from sepsis, and IFN-γ, IL-1β, and IL-6 have a protective effect by decreasing the PCI production by hepatocytes.

Disclosure of Interest: None declared.

PO285-TUE

Does the coagulation cascade play a role in retinal pathologies?

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Background: Very little is known about the involvement of the coagulation cascade and its correlation to inflammation in retinal pathologies.

Aims: To evaluate and correlate the coagulation and inflammation cascades within the vitreous substance of patients with vitreo-retinal (VR) pathologies.

Methods: Vitreous samples were collected from 91 patients. VR pathologies included macular holes (MH), epiretinal membranes (ERM), rhegmatogenous retinal detachments (RRD), vitreous hemorrhages (VH), and tractional retinal detachments (TRD). Pathologies were grouped to three main categories: 1. MH and ERM (*n* = 29); 2. RRD (*n* = 33); 3. VH and TRD (*n* = 29). Key proteins in coagulation and inflammation, thrombin anti-thrombin (TAT) and iInterleukin-6 (IL-6) were assessed by ELISA, respectively.

Results: TAT and IL-6 levels were found highest in RRD followed by VH and TRD and lower in patients with ERM and MH. TAT levels were significantly higher in the TRD/VH and RRD compared to the MH/ERM group. No significant difference was found between the TRD/VH and RRD groups. IL-6 levels were significantly different in the MH/ERM group and TRD/VH and RRD groups, as well as between TRD/VH and RRD. Linear correlation between IL-6 and TAT was found in the MH/ERM group. The RRD group showed a trend towards a linear correlation, while the TRD/VH group did not show any correlation.

Conclusion: Our study demonstrates that TAT levels were significantly higher within the disease groups compared with the control, but not statistically different between the disease groups. Correlation between TAT and IL-6 levels were not uniform in the different VR pathologies. The insight into the basic pathophysiological processes leading to disrupted retinal function may pave the way to the development of novel

therapeutic interventions for the treatment of deleterious ophthalmic diseases. Future studies will aid in confirming the role of the coagulation system in different retinal pathologies.

Disclosure of Interest: None declared.

PO286-TUE

Clinical presentation of thromboembolic events in inflammatory bowel disease

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Background: Patients with inflammatory bowel disease (IBD) have a higher risk to develop thromboembolic events (TE) compared to the healthy population. TE has a substantial impact on mortality in IBD and TE prevention requires improved awareness and management.

Aims: To describe the clinical characteristics of IBD patients with a history of TE; focusing on recurrence of TE, disease activity at time of TE, IBD medication at time of TE and surgery before TE.

Methods: In a retrospective monocentric cohort study, we included all IBD patients in which a TE, arterial or venous, occurred between January 1987 and March 2014. Disease activity was defined based on physician's global assessment. Patients with a TE before IBD diagnosis were excluded.

Results: 84 IBD patients with a history of TE (63% Crohn's disease, 4% males) and a mean age of 45 ± 15 years were included, whereof 25/84 (30%) patients were identified with recurrent TE. 70/84 (83%) developed a venous TE, with a deep vein thrombosis (DVT) as the major manifestation (28/70, 40%) followed by a pulmonary embolism (PE) (16/70, 23%). At time of TE, 60/84 (71%) patients were diagnosed with active disease. Thirty-six percentage patients were on steroid therapy, 23% on 5-amino salicylic acids, 23% without specific IBD treatment, 18% on azathioprine, 12% on biological therapy, 5% on methotrexate and 4% on antibiotics. Moreover, within a 6-month period preceding the TE, 31/84 (37%) underwent surgery and 33/84 (40%) received steroid treatment.

Conclusion: With this descriptive study we confirm the association between disease activity and the occurrence of TE. Venous TE occurred more than arterial TE and a substantial number of patients had additional risk factors such as recurrence of TE or recent surgery. Thirty-six percentage received steroid therapy at time of TE and 40% used steroids in a 6 months period preceding the TE.

Disclosure of Interest: None declared.

PO287-TUE

Loss of PAR-4 receptor affects renal ischemia reperfusion in mice

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Background: Protease-activated receptor 4 (PAR-4) is a G-coupled receptor that can be activated upon proteolytic cleavage by serine proteases such as thrombin. A variety of cell types such as endothelial cells, epithelial cells, platelets and leukocytes express PAR-4. Agonists of PAR-4 have been shown to induce leukocyte rolling and adherence, suggesting a pro inflammatory role for this receptor. Activation of coagulation, characterized by thrombin generation, is a hallmark of renal ischemia/reperfusion (I/R) injury. The influx and activation of granulocytes in renal tissue plays a crucial role in the pathology of

renal I/R injury. The role of PAR-4 in the pathology of acute I/R injury has not been studied so far.

Aims: Here we investigated the effect of PAR-4 deficiency on renal I/R inflammation and injury.

Methods: Wild type (WT) and PAR-4 knockout mice were subjected to unilateral renal I/R. Mice were sacrificed after 24 h and kidneys were collected. Histopathological scoring was performed on PAS-D-stained renal tissue sections. Granulocytes were stained with ly6G mAb and the number of positive cells was counted within 10 non-overlapping fields ($\times 400$). Renal expression of kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) was determined by qPCR on renal tissue homogenates.

Results: PAR-4 knockout mice showed lower amounts of renal tissue damage, as determined by semiquantitative histological scoring, accompanied by significantly lower numbers of infiltrating granulocytes than WT mice. Compared to WT mice, PAR-4 knockout mice displayed more tubular luminal protein casts, predominantly located in the renal cortex. mRNA levels of KIM-1 and NGAL were comparable between WT and PAR4 knockout animals.

Conclusion: Our data show that PAR-4 plays a role in the pathology of renal I/R injury, affecting renal tissue damage and the inflammatory response. Further research will be needed to elucidate which cell types are responsible for the observed phenotype.

Disclosure of Interest: None declared.

PO288-TUE

TIPE2 is induced by simulated microgravity in monocytes

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Background: Microgravity is associated with several health issues including dysregulation of immune function. A growing body of evidence shows that tumor necrosis factor- α -induced protein 8-like 2 (TIPE2) plays a important role in immune homeostasis, suggesting that TIPE2 might be regulated by microgravity, verification of the mechanisms by which microgravity interacts with immunity cells may enable development of a promising strategy and validate some necessary countermeasures for astronauts.

Aims: To investigate the effect of simulated microgravity on TIPE2 and underlying mechanism in monocyte.

Methods: THP-1 cells were cultured under a simulated microgravity environment using the rotating cell culture system (RCCS). The TIPE2 mRNA and protein, Egr-1, I κ B and nuclear factor κ B (NF- κ B), were analyzed using quantitative PCR and western blotting, levels of IL-6 and IL-1b in culture cells were detected by ELISA, respectively.

Results: TTPE2 induction was confirmed by RT-PCR and immunoblotting demonstrating a more than 16-fold ($P < 0.001$) increase in TIPE2 mRNA in THP-1 cells cultured in simulated microgravity compared with static controls, and a consistent protein upregulation. Egr-1 transcription factor was increased in microgravity via PKC and ERK1/2 MAPK. Furthermore, inflammatory cytokines (IL-6 and IL-1b) and NF- κ B were upregulated in microgravity-activated cells.

Conclusion: TIPE2 was induced in microgravity, PKC, ERK1/2 MAPK and NF- κ B may be relevant target in immunity and inflammation for microgravity.

Disclosure of Interest: None declared.

PO289-TUE

Mouse macrophages upregulate podoplanin under inflammatory and apoptotic conditions

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Background: Podoplanin (PDPN), the endogenous ligand of CLEC-2, is expressed at high levels on lymphatic endothelial cells, kidney podocytes and lung type 1 alveolar cells. The interaction of CLEC-2 on platelets with PDPN on lymphatic endothelial cells is crucial for the normal development of the lymphatic system. Recently, PDPN was shown to be expressed on inflammatory macrophages. Accordingly, PDPN-expressing macrophages interact with CLEC-2 and induce platelet activation and aggregation. In turn, the binding of CLEC-2 may regulate activation of macrophages.

Aims: The aim of this study is to investigate under which conditions mouse macrophages and monocytes upregulate PDPN expression focussing on inflammatory stimuli and apoptosis. The consequence of this interaction was investigated.

Methods: Using mouse macrophages cell line RAW264.7 and bone-marrow derived macrophages and monocytes, we investigated the conditions that lead to expression of PDPN by quantitative PCR and flow cytometry.

Results: PDPN was absent from RAW267.4 macrophages as shown using PDPN specific antibody and by PCR. LPS-treated cells as well as apoptotic macrophages (induced by staurosporine and cycloheximide) upregulate PDPN expression by 10.7 ± 3.2 fold and 9.2 ± 2.9 fold (Mean $\pm$ SEM, $n = 5$), respectively. PDPN expression was observed at the mRNA level as measured by q-PCR, with 2410 ± 567.6 fold increase for LPS-treated macrophages and 1888 ± 1137 fold increase for apoptotic macrophages ($n = 4$). In addition, PDPN upregulation was observed on bone marrow-derived macrophages treated with LPS or apoptotic macrophages. Under the same conditions, monocytes or the mouse endothelial cell line (sEnd-1) did not show an increase in PDPN expression.

Conclusion: Under inflammatory conditions, mouse macrophages upregulate PDPN mRNA level and PDPN expression on macrophages. The presence of PDPN on inflammatory and apoptotic macrophages may contribute to reciprocal activation of platelets and regulation of thrombus formation and inflammatory processes.

Disclosure of Interest: None declared.

PO290-TUE

Anti-inflammatory effect of non-canonical peptide-agonist of PAR-1 on rat mast cells resembles activated protein C action

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Background: It's known that activated protein C (APC) and thrombin cleave PAR-1 and demonstrate opposite physiological effects, which was described on endothelial cells as "biased agonism" (Mosnier et al., 2012).

Aims: Estimation the impact of peptide, analogous to tethered ligand of PAR1 released by APC, on secretion of histamine from mast cells (MC) in an *in vitro* inflammation model and on the neuronal survival in coculture of neurons with activated MC.

Methods: We analyzed secretion of histamine from activated rat peritoneal MC (modified from Shore et al., 1959); the survival of neurons was estimated using fluorescent dyes.

Results: TNF α (50 ng mL⁻¹) induces activation of MC and increase of histamine release by 16.4% compared with control. Pretreatment of the activated MC by 10 mM Pep9 reduces histamine release by 18%, and by 10 nM APC – by 19% compared to TNF α -activated cells. It was found that Pep9 (0.1–10 mM) effectively suppresses histamine release from activated MC. Histamine release in the groups of cells treated by Pep9 or APC have no significant differences from the control values. Obtained data indicate that anti-inflammatory effect of Pep9 is similar to APC. Analysis of neuronal survival at co-cultivation with MC, activated by TNF α or lipopolysaccharide, showed a significant decrease the number of living neurons in culture (1.4- and 2 times, respectively), and increase the number of apoptotic cells (1.3- and 1.6-fold, respectively), which indicates a developing neuroinflammation. Pretreatment of MC with Pep9 or APC increased neuronal survival and decreased apoptosis of neurons. Thus, the Pep9 causes the decrease of MC's secretion resulting in neuronal survival increase and downregulation of inflammation.

Conclusion: We discovered previously unknown anti-inflammatory effect of Pep9 which is similar to APC on MC. Apparently, this peptide could be targeted as a potential therapeutic agent at neuroinflammation and other inflammatory diseases. This work was supported by MES RF grant (№14.604.21.0017).

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PO291-TUE

High-fat diet is associated with increased von willebrand factor-related procoagulant activity in human and murine models

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Background: Obesity is a chronic inflammatory state that is well documented to be associated with an increased risk of thrombosis. Among the procoagulant influences induced by obesity is an increase in plasma von Willebrand factor (VWF) levels. Thus, in obese states, elevated VWF antigen (VWF:Ag) levels represent an independent risk factor for thrombosis. VWF is an acute-phase glycoprotein that links inflammation with increased procoagulant activity in the process of immunothrombosis.

Aims: We investigated the influence of high-fat diet (HFD) on the VWF-associated immunothrombosis in human and murine models.

Methods: Normal C57BL/6 littermates were fed either a HFD (60% fat) or standard diet (STD) (10% fat) and blood was collected after 2 & 10-weeks. At 10 weeks, arterial thrombus formation was observed with an intravital laser-induced murine cremaster arteriole injury model using photoactivated hematoporphyrin. Human subjects fasted for 12 h and were fed a high-fat (52%) or low-fat (8%) meal.

Results: After 2 weeks, HFD-fed mice had higher VWF:Ag (1.2-fold, $P = 0.04$), FVIII activity (FVIII:C) (1.4-fold, $P = 0.005$), the inflammatory marker, cell-free DNA (CF-DNA) (1.11-fold, $P = 0.03$), total cholesterol (1.4-fold, $P = 0.002$) and lower platelet counts (0.8-fold, $P = 0.04$) compared to STD-fed mice. VWF:Ag (1.4-fold, $P < 0.0001$), CF-DNA (1.14-fold, $P = 0.007$) and cholesterol (1.4-fold, $P = 0.01$) continued to increase after 10 weeks. The increased VWF-mediated procoagulant activity in HFD-fed mice was associated with a 0.7-fold shorter arterial occlusion time than STD, in an intravital thrombosis model ($P = 0.02$). Human samples collected 5 h post high-fat meal, had significantly lower ADAMTS13 activity (0.8-fold, $P = 0.01$) than when fed a low-fat meal. Changes to VWF:Ag and FVIII:C were not significant.

Conclusion: Inflammation associated with high-fat diet increases VWF-related prothrombotic activity and may contribute to an increased risk of immunothrombosis in obese individuals.

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PO292-TUE

Inflammatory and endothelial markers and their relations to the haemostatic potential during the menstrual cycle

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Background: Total thrombin concentration was previously shown to be higher during the luteal phase of a menstrual cycle. This was not a result of concurrent changes in coagulation factors.

Aims: Since the inflammation and the coagulation systems interact closely and thrombin has a major influence on the inflammatory response, the present report studies inflammatory and endothelial markers in relations to haemostasis as a possible explanatory mechanism for thrombin generation variation during the menstrual cycle.

Methods: The study cohort consisted of 102 healthy women not taking hormonal medications. Blood samples were collected in the early follicular and the late luteal phase. The inflammatory and endothelial markers studied were pentraxin-3 (PTX-3), high sensitivity CRP, sE- and sP-selectin, intracellular and vascular cell adhesion molecules and cathepsins L, B and S. The haemostatic parameters analysed were factors II, VII, VIII, X and von Willebrand, fibrinogen, D-dimer, plasminogen activator inhibitor-1 and antithrombin. Thrombin generation was assessed by the Calibrated Automated Thrombogram[®] assay. Estradiol, progesterone, anti-müllerian hormone and sex-hormone binding globulin were also measured. We used the paired t-test to study the differences between the biomarkers during the two phases ($P < 0.05$), as well as multiple regression and correlation analysis (Pearson's) for the associations between markers.

Results: No significant associations between coagulation and endothelial and vascular markers were observed. PTX-3 and hs-CRP were significantly higher during the follicular phase ($P < 0.001$ respectively $P = 0.025$). The other inflammatory and endothelial markers, with the exception of cathepsin B, were higher, albeit not significantly, during the follicular phase.

Conclusion: Increased inflammatory activity was observed during the early follicular phase, which corresponds to the inflammatory described during menstruation. This increase was independent of haemostatic or hormonal changes.

Disclosure of Interest: None declared.

PO293-TUE

Cigarette smoking significantly reduces gen- and protein expression of the pro-inflammatory cytokine IL-12

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Background: Interleukin-12 (IL-12) is encoded by two separately located genes; IL-12p35 and IL-12p40, and the bioactive heterodimer (IL-12p70) is a central cytokine in the immune response. It is mainly produced in monocytes and macrophages and induces interferon gamma (INF γ), and *vice versa*. IL-12 is critical in the initiation and progression of pro-inflammatory T helper cell 1-type responses. Circulating IL-12 has been shown to be elevated in atherosclerosis, however, also reported to be down-regulated in different cell types by cigarette smoking.

Aims: To investigate the influence of cigarette smoking on circulating IL-12 levels in patients with coronary artery disease (CAD). Gene-expression of IL-12p35, IL-12p40 and INF γ in circulating leukocytes was further analysed to explore potential regulatory mechanisms.

Methods: Thousand patients with angiographically verified stable CAD were investigated (203 current smokers, 78% men, mean age 62 years). Informed consent was obtained from all and the study was approved by the Regional Ethics Committee. Circulating IL-12 was measured in all by the ELISA kit KHC0121 (Invitrogen), whereas gene-expression was analysed in 210 randomly included patients (43 current smokers) from the total cohort, by real-time PCR and Taq-Man assays (Applied Biosystems).

Results: Circulating IL-12 levels and expression of the IL-12p35 gene was considerably lower in current smokers vs. non-smokers ($P < 0.001$, $P = 0.025$, respectively) and INF γ gene-expression was 14% lower ($P > 0.2$). The IL-12p40 gene was not expressed in circulating leukocytes.

INF γ gene-expression was positively correlated to IL-12p35 gene-expression and circulating IL-12 ($r = 0.33$, $P = 0.031$ and $r = 0.50$, $P = 0.001$, respectively), only in current smokers.

Conclusion: We observed a modified immune-response in current smokers, shown by reduced gene- and protein expression of IL-12. The influence of cigarette smoking on IL-12, its intra-cellular signalling and regulatory mechanisms needs to be further explored.

Disclosure of Interest: None declared.

PO294-TUE

The interaction between expression of coxsackie-adenovirus receptor on the surface of platelets and cardiomyocytes, inflammation and virus persistence inside cells

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Background: The coxsackie and adenovirus receptor (CAR) was initially identified as a receptor for coxsackie B and adenoviruses, which is known to promote virus myocarditis. It can not only mediate virus entry but its physiological role is to be a part of intercellular communication

Aims: to evaluate the interaction of virus persistence in platelets in cardiomyocytes (CM), CAR expression on cells surface and the activeness of inflammation

Methods: CAR expression was investigated in 23 pts with suspected myocarditis (group 1) and in 38 pts with arrhythmias (group 2) and 12 healthy subjects (HS). Diagnosis in group 1 was confirmed by endomyocardial biopsy (EMB). CAR expression was analyzed by immuno-

fluorescent and immunohistochemistry method. The virus persistence was estimated by PCR. The level of tumor necrosis factor α (TNF α), interleukin 6 (IL6), transforming growing factor β 1 (TGF) was estimated by ELISA.

Results: From 0.2 to 6.4% platelets were CAR-positive in HS. CAR-expression on platelets was much higher in pts than in HS (4.5 [2.0;8.9]% for group 1, $P = 0.039$), 8.0 [4.0;19.0]%, $P = 0.0002$ for group 2. The only virus detected in 39% EMB samples was parvovirus B19. Virus persistence, the level of CAR expression on platelets and CM was not associated with activeness of inflammation in myocardium. Pts with increased number of CAR-positive platelets were characterized by increased level of IL6 and TNF α . CAR expression on platelets showed 64.3% sensitivity and 66.7% specificity in detection of myocardial inflammation. The grade of CAR expression on CM was much higher in pts with increased level of IL6 (2.5 ± 0.55 vs. 2.0 ± 0.43 , $P = 0.036$).

Conclusion: CAR expression in myocardium is associated with increased level of IL6 and doesn't depend on activeness of inflammatory infiltration. There was no relation of CAR expression and virus persistence inside cells. According to its sensitivity and specificity we propose using CAR expression on platelets a marker in detection of systemic inflammation.

Disclosure of Interest: None declared.

PO295-TUE

The role of inflammation in pregnancy-related venous thrombosis

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Background: The relationship between inflammation and coagulation is well established, and inflammation is associated with arterial thrombosis. The relationship between inflammation and venous thrombosis (VT) remains unclear.

Aims: To investigate whether patients with pregnancy-related VT have elevated inflammatory markers compared to controls.

Methods: The present study included 313 women with pregnancy-related VT during 1990–2003 and 353 controls. All women donated a single blood sample and answered a questionnaire in 2006. In this study, we excluded women who were pregnant or were using anticoagulants or oral contraceptives at the time of blood sampling. The final study population comprised 282 women with pregnancy-related VT and 315 controls. As possible predictors of pregnancy-related VT we included the inflammatory markers high sensitivity CRP, interleukin (IL)-6, IL-8, IL-10, monocyte chemoattractant protein (MCP)-1, and the two adhesion molecules intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1. We used the >90th percentiles among controls as cut-offs.

Results: The cases were younger, had higher body mass index (BMI), and were more often smokers compared to controls. Sixty-eight percentage of the cases had deep vein thrombosis (DVT) in a lower limb, 24% had pulmonary embolism, and 8% had DVT outside the lower limbs or lungs. Fifty-one percentage of VTs occurred postpartum. In univariate analysis IL-6, IL-10, ICAM-1, and VCAM-1 were significantly associated with VT. When adjusting for age, body mass index, and smoking, only VCAM-1 above the 90th percentile was independently associated with VT with an adjusted odds ratio of 3.1 (95% CI 1.8–5.0, $P < 0.001$).

Conclusion: Women with a history of pregnancy-related VT had elevated VCAM-1 many years after the event as compared to a control group. This may be due to systemic inflammation.

Disclosure of Interest: None declared.

PO296-TUE

The Extracellular Fibrinogen-binding protein (Efb) secreted by *Staphylococcus aureus* binds p-selectin and competitively inhibits its interaction with p-selectin glycoprotein ligand-1 (PSGL-1).

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Background: *Staphylococcus aureus* releases a variety of extracellular factors that participate in the infection induced by this bacterium. Extracellular fibrinogen-binding protein (Efb) is released by *S. aureus* and inhibits platelets both *in vitro* and *in vivo*. In addition, Efb reduces immune response and exacerbates the infections by *Staphylococcus aureus*.

Aims: In order to identify the molecular mechanisms underlying the pathogenic and anti-haemostatic role of Efb, we aimed to identify platelet proteins directly interacting with Efb.

Methods: Efb-interacting proteins within platelets were pulled down by sepharose-Efb beads and identified by mass spectrometry. Secondly, proteomics results were confirmed by sepharose-Efb pull-down followed by immunoblotting using antibodies specific for the proteins identified by mass spec. Finally, in order to determine which proteins are responsible for mediating the interaction of Efb with platelets, we performed flow cytometry experiments with fluorescein-labelled Efb in the presence of specific inhibitory antibodies. By using truncation mutants, the Efb domains responsible for interacting with different interacting proteins were identified.

Results: We identified a series of platelet proteins able to interact with Efb. Amongst these proteins, integrin α IIB β 3 and P-selectin are expressed on platelet surface and play important biological roles. Both interactions were confirmed by immunoblotting. The interaction with P-selectin is direct (i.e. detected in the absence of fibrinogen) and the N-terminal domain of Efb is responsible for interacting with P-selectin. Using a co-immunoprecipitation approach, we discovered that Efb inhibits the P-selectin/PSGL-1 interaction by directly binding P-selectin.

Conclusion: The inhibition of the P-selectin/PSGL-1 interaction by Efb may underly the ability of this bacterial protein to exacerbate of *S. aureus* infection. Efb may in fact interfere with platelet-leukocyte complex formation and leukocyte extravasation.

Disclosure of Interest: None declared.

Innate and acquired Immunity

PO297-TUE

Critical behavior of subcellular density organization during neutrophil activation and migration

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Background: Physical theories of active matter continue to provide a quantitative understanding of dynamic cellular phenomena, including cell locomotion. Although various investigations of the rheology of cells have identified important viscoelastic and traction force parameters for use in these theoretical approaches, a key variable has remained elusive both in theoretical and experimental approaches: the spatiotemporal behavior of the subcellular density.

Aims: To elucidate the subcellular density dynamics in neutrophils undergoing chemokinesis following uniform bacterial peptide stimulation using the established, quantitative imaging approach, non-interferometric quantitative phase microscopy (NIQPM).

Methods: Human polymorphonuclear leukocytes were purified from venous blood, incubated on fibronectin-coated glass coverslips, and exposed to the bacterial peptide, formyl-Met-Leu-Phe. Neutrophils were then fixed with 4% paraformaldehyde every 20 s. Fixed neutrophils were permeabilized with methanol and stained with anti-myosin light chain2 pSer19 (3675) and anti-actin in blocking buffer overnight. Secondary antibodies conjugated with AlexaFluor 488 and TRITC phalloidin in blocking buffer were added and incubated for 2 h in the dark. Neutrophil subcellular density was quantified using the NIQPM imaging techniques and cell area, height, and cell volume were determined using a custom MATLAB program.

Results: We determined that subcellular density decreases in a power-law dependent fashion as neutrophils become activated, and that a critical density is reached when neutrophils polarize and begin chemokinesis. It was also identified that a continuum law of mass conservation that links area, mean density, and total dry mass holds for resting, activated and motile neutrophils.

Conclusion: Our approach and quantitative findings will enable investigators to define the physics coupling cytoskeletal dynamics with subcellular density dynamics during cell migration.

Disclosure of Interest: None declared.

PO298-TUE

Naturally occurring antibodies to oxidation-specific epitopes in patients with venous thromboembolism

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Background: Naturally occurring IgM antibodies to oxidized LDL (OxLDL) protect from atherosclerosis. OxLDL has proinflammatory and prothrombotic properties by expressing oxidation-specific epitopes (OSE). A subset of circulating microparticles carries the same OSE also present in OxLDL. Thus, humoral immune responses to OSE of OxLDL may be involved also in the pathogenesis of venous thromboembolism (VTE).

Aims: To evaluate the role of antibodies against OSE in VTE.

Methods: We followed patients with a first unprovoked VTE after anticoagulation and excluded those with cancer, pregnancy, long-term antithrombotic therapy, or major thrombophilia. Study end point was recurrent VTE. Levels of IgM antibodies to copper-OxLDL in plasma were determined by chemiluminescent ELISA. The effect on the recurrence risk and the impact of competing events were assessed using time-to-event models. Anti-OxLDL IgM levels are given as RLU/100 ms (median, 25th, 75th percentiles). The study was approved by an ethics committee and patients' consent was obtained.

Results: Anti-OxLDL IgM levels were 20.2 (12.3, 34.7) and correlated with sex (lower in men) and recurrence (lower in recurrence). 151 (22.8%) of 663 patients (mean age 48 years) had recurrence during a median follow up of 67 months. For each doubling of anti-OxLDL IgM levels, the hazard ratio (HR) of recurrence was 0.84 (95% CI 0.72–0.97; $P = 0.02$). The probability of recurrence after 5 years in patients stratified according to anti-OxLDL IgM was 12.4% (95% CI 8.4–17.2%) in the group with levels higher than 26.39 compared to 20.0% (95% CI 16.1–24.1%) among those with lower levels ($P < 0.01$). The corresponding HR was 0.56 (95% CI 0.33–0.94; $P < 0.01$) for higher anti-OxLDL IgM and was 0.68 (95% CI 0.47–0.98; $P = 0.04$) after adjustment for sex.

Conclusion: Our data demonstrate a role for OxLDL-specific IgM in venous thrombotic disease. Patients with low anti-OxLDL IgM levels are at higher risk of thrombosis while those with high levels are protected.

Disclosure of Interest: None declared.

PO299-TUE

C. canimorsus affects coagulation by proteolytic cleavage of factor X

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Background: *Capnocytophaga canimorsus* is a Gram-negative bacterium belonging to the oral flora of dogs. Transmission to humans occurs via bites or scratches. Despite being only weakly pro-inflammatory, *C. canimorsus* often causes fulminant septicemia, commonly accompanied by disseminated intravascular coagulation (DIC).

Coagulation is part of the innate immune response. The entrapment of bacteria in blood clots promotes a more efficient elimination. However, a range of bacteria have developed strategies to interfere with this mechanism.

Aims: In order to elucidate what contributes to the pathogenesis of *C. canimorsus* infection, considering the frequent occurrence of bleeding abnormalities, our aim was to find out if *C. canimorsus* interferes with coagulation.

Methods: We incubated normal pooled plasma (NPP) with *C. canimorsus* strain 5 (Cc5), monitored thrombin generation by a calibrated automated thrombogram (CAT) assay and measured PT and aPTT. Coagulation factor activity in Cc5 treated plasma was assessed using factor depleted plasmas. We performed ¹²⁵I labeling of FX to investigate its cleavage in NPP. Irreversible inhibition of bacterial serine proteases was achieved by treatment of Cc5 with 1 mM AEBSF prior to experiments with NPP.

Results: We observed that Cc5 blocked thrombin generation in a dose dependent manner. PT and aPTT of NPP incubated with Cc5 were significantly increased. Amongst the panel of clotting factors tested, we observed a strong impairment of FX activity. We found that Cc5 cleaved FX in plasma and that this was inhibited when bacteria had been treated with AEBSF. In addition, AEBSF treatment of Cc5 completely reversed the increase of PT and aPTT.

Conclusion: *C. canimorsus* 5 degrades FX and inhibits coagulation. We are currently investigating the site of FX cleavage and trying to identify the bacterial protease that mediates the cleavage. It is thinkable that, by blocking coagulation, Cc5 could spread more easily and possibly also aggravate DIC associated bleeding.

Disclosure of Interest: None declared.

PO300-TUE

Polyphosphate acts as a cofactor for C1-inhibitor-mediated regulation of the classical pathway of complement

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Background: Polyphosphate (polyP) is a linear polymer of orthophosphate units that is found in all living organisms. In humans, polyP is abundant in platelet-dense granules and mast cell granules, suggesting a modulatory role in hemostasis and inflammation. Complement is activated via the classical (CP), alternative (AP) or lectin (LP) pathways, converging at the terminal pathway to form the membrane attack complex. We previously showed that polyP destabilizes the C5b,6 components of the terminal pathway (Wat. et al., Blood 2014). Interestingly, PolyP also binds to C1-inhibitor (C1INH), the serpin

modulator of C1s, the CP-specific initiating protease. This raises the possibility that polyP may act as a cofactor of C1INH and thus act to regulate the classical pathway via this mechanism.

Aims: To test whether polyP alters the modulatory function of C1INH in the CP.

Methods: Binding of polyP to C1s and C1INH was measured using surface plasmon resonance (SPR). The inhibitory effect of polyP on C1s-mediated cleavage of the CP proteins C4 and C2 was analyzed using SDS-PAGE. The overall effect of polyP was tested by measuring the deposition of the CP degradation fragment C4d on choroidal endothelial cells (CEC) using FACS.

Results: PolyP bound to both C1s and C1INH with K_d values of 400 and 33 nM, respectively. The rate of association between C1s and C1INH was increased over 1000-fold in the presence of polyP, indicating that it does act as a cofactor for the C1s-C1INH interaction. PolyP enhanced C1INH inhibition of C1s-mediated cleavage of C4 and C2 in a size- and concentration-dependent manner. C4d deposition on CEC was significantly suppressed by polyP, supporting the biochemical evidence that polyP inhibits initial activation of the CP.

Conclusion: PolyP acts as a cofactor for C1INH and thus regulates complement activation at multiple steps of the cascade. This study indicates that polyP is an important regulator for this key pro-inflammatory mechanism.

Disclosure of Interest: None declared.

PO301-TUE

Human monocyte subsets differently express tissue factor

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Background: Monocytes play a key role in inflammation and coagulation. Circulating monocytes can be divided into three subsets using their distinct surface expression of CD14 and CD16, namely as 'classical monocytes' (CM, CD14 + +CD16-), 'intermediate monocytes' (IM, CD14 + +CD16 +) and 'non-classical monocytes' (NCM, CD14 + CD16 ++).

Aims: We hypothesized that besides their known characteristic inflammatory potential, monocyte subtypes exhibit specific coagulatory profiles.

Methods: Fifty consecutive patients admitted to a medical ICU at a tertiary care center and 30 hospital control patients were enrolled and blood was taken at admission for the measurement of monocyte-subset-specific tissue factor (TF) expression. Furthermore, whole blood obtained from healthy controls was stimulated with TNF- α and LPS for 4 h and TF-expression was measured. Moreover, subset specific TLR4 and TNFR expression was measured in blood samples from healthy donors.

Results: At ICU admission, IM (529 $\pm$ 272; MFI) showed significantly higher TF expression as compared to CM (369 $\pm$ 79; $P < 0.0001$), while NCM showed the lowest TF expression (197 $\pm$ 104; $P < 0.0001$ compared to CM and IM). Interestingly, TF expression by IM was significantly higher in ICU patients than in hospital controls ($P < 0.05$), whereas CM and NCM showed no difference. CM and IM from healthy controls showed no difference in TF expression. However, incubation with LPS and TNF- α showed a significantly higher increase of TF surface expression in IM, when treated with LPS, as compared to CM and NCM ($P < 0.05$). In contrast, the response to TNF- α was comparable between all three subtypes. Measurement of the respective receptors TLR-4 and TNF-R revealed highest TLR-4 expression in IM while TNF-R expression was more balanced between subsets.

Conclusion: Beside their well-characterized inflammatory phenotype, monocyte subtypes also show a distinct coagulatory phenotype with

highest TF expression in IM, medium TF expression in CM and lowest TF expression in NCM.

Disclosure of Interest: None declared.

PO302-TUE

MHC class I antigen presentation by megakaryocytes

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Background: Megakaryocytes are the progenitor cells of platelets in bone marrow. In addition to their role in hemostasis, several studies have previously shown that there is an immunological function played by platelets. Platelets have been shown to process extra-cellular antigens and present in the context of major histocompatibility complex (MHC) class I (cross presentation). Classically, MHC class I display endogenous antigens on the cell surface, whereas cross-presentation characterizes the mechanism of generation of peptide ligands from exogenous proteins. However, the immunological role observed in platelets has not been assessed in their parent cells, the megakaryocytes. To ascertain the ability of megakaryocytes to actively present exogenous antigens and trigger an immune response would help to unravel the possible role of megakaryocytes in immunity and in immune diseases, such as immune thrombocytopenia (ITP).

Aims: Our aim is to show that megakaryocytes are able to process and present foreign antigens in the context of MHC class I.

Methods: Megakaryocytes were cultured from mouse C57BL/6 bone marrow and pulsed with fluorescent ovalbumin (OVA). Immunofluorescence by confocal microscopy was used to assess the processing and cellular localization of OVA. Several markers belonging to endocytosis and antigen presentation pathways, as well as an antibody against the ovalbumin octapeptide SIINKEFL in association with MHC class I molecules were used.

Results: OVA was internalized by megakaryocytes and colocalized with secretory granule markers. The OVA octapeptide SIINKEFL/MHC class I complex was shown to be stored in granule-like structures and presented at the plasma membrane.

Conclusion: Mouse megakaryocytes can readily uptake extra-cellular proteins and degrade them into peptides. These peptides can then be loaded on MHC class I molecules and stored in granule-like structures. However, the exact nature of these structures remains to be determined, as well as the immune functionality of this presentation.

Disclosure of Interest: None declared.

PO303-TUE

Prothrombin mutants reveal a new mechanism of MASP-1 mediated clotting

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Background: We have recently shown that mannan-binding lectin-associated serine protease-1 (MASP-1) enhances the ability of plasma and whole blood to clot. This reaction proved to be prothrombin-dependent, and the effects of MASP-1 on clotting added up with the effects of thrombin.

Aims: The aim of this study was to reveal the detailed mechanism of MASP-1 mediated clot formation by analysing the cleavage of prothrombin (PT) mutants.

Methods: We used two existing PT mutants with altered cleavage sites (R271Q, R320Q). In addition, we expressed the novel mutant PT R393Q in HEK293 cells and purified the protein by ion exchange chromatography. We incubated MASP-1 with PT wildtype or individual mutants and analysed the cleavage products at different time points by SDS-PAGE and N-terminal sequencing. Hirudin, a specific thrombin inhibitor which does not inhibit MASP-1, was used to suppress thrombin-mediated cleavage steps.

Results: MASP-1 cleaves wildtype PT at the three sites R271, R320 and R393. By incubation of MASP-1 with the three PT mutants, we could show that MASP-1 uses two routes of PT activation, starting with cleavage at R271 or at R393. Cleavage at R271 generates prethrombin-2, while cleavage at R393 leads to a 'meizothrombin analogue' (mIIR393). Prethrombin-2 gets further cleaved by MASP-1 at R393 forming a 'β-thrombin analogue' (β-thrombin). On the other route, mIIR393 cleaves itself at R320 or at R271 (generating mIIR393' or β-thrombin respectively). Cleavage experiments in the presence of hirudin support the findings that the arising intermediates are thrombin-species which are involved in back-cleavage of PT and some intermediates.

Conclusion: We have shown that MASP-1 is able to mediate clotting by alternative PT cleavage that uses two different routes of activation. These routes give rise to three putative new thrombin species (β-thrombin', mIIR393, mIIR393') of which β-thrombin' and mIIR393 affect the coagulation cascade.

Disclosure of Interest: None declared.

PO304-TUE

Complement and tissue-factor induced thrombin generation in the pathogenesis of venous thromboembolism

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Background: Venous thromboembolism (VTE) appears to initiate most often in valve pockets of large veins with a local milieu characterized by stasis and severe hypoxia. This may lead to activation of the complement system, which can trigger tissue factor (TF)-induced coagulation. A comprehensive cross-talk between the complement- and coagulation systems has emerged during the last years. No study has investigated the role of the complement system in the pathogenesis of VTE.

Aims: To investigate whether pathways of the complement system are associated with TF-induced thrombin generation and VTE risk.

Methods: A case-control study was performed in 24 patients with unprovoked VTE and 24 age- and sex-matched controls recruited from the general population. TF-induced thrombin generation was measured in plasma using the CAT-assay. Serum complement activity of the classical-, alternative- and lectin pathways was measured by the Wieslab® enzyme-immunoassay and MBL was quantified by ELISA. Odd ratios (OR) with 95% confidence intervals were calculated. The study was approved by the research ethics committee and all subjects gave informed written consent.

Results: The VTE patients had shortened TF-induced lag-time (4.8 min (4.5–5.2) vs. 5.8 min (4.8–8.0), $P < 0.001$) (median with interquartile range) (OR 0.3, 95% CI: 0.1–0.6) and higher total thrombin generation (ETP) (1383 nM*h (1220–1507) vs. 1264 nM*h (1103–1416) than controls (OR 1.7, 95% CI: 1.0–3.7). Low lectin pathway

activity, verified to be due to MBL deficiency, was associated with a 3.5-fold higher (95%CI: 0.8–15.3) VTE risk, whereas subjects with activity in the highest quintile of the classical pathway had a 4.5-fold (95% CI: 0.8–24.7) higher VTE risk. There were no interrelations between parameters of TF-induced thrombin generation and deviations of the MBL and classical pathways of complement.

Conclusion: Our findings may imply that deviations in lectin and classical pathways of the complement system might be associated with risk of unprovoked VTE.

Disclosure of Interest: None declared.

PO305-TUE

Unique genetic variation in an invasive serotype M23ND strain of *Streptococcus pyogenes* influences bacterium–host cell interactions and complement-mediated opsonization

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Background: The complement and coagulation pathways act in concert to prevent pathogenic bacterial infections. *Streptococcus pyogenes*, or Group A streptococcus (GAS), is a human specific pathogen that exploits both host complement and fibrinolytic systems to facilitate its survival and dissemination within the host. The remarkable genetic variability present within GAS species results in the differences in the pathogenic potential between strains. Therefore, identification and characterization of different GAS isolates have been central to pathogenic studies of GAS infections.

Aims: To elucidate the influence of genetic variation on virulence of Serotype M23 (*emm23*) strain (M23ND), isolated from an invasive human infection.

Methods: Molecular biology techniques were employed to modify isolated M23ND/covR+S– strain into an M23ND isogenic strain with a native covS mutation corrected to M23ND/covR+S+. Two well-characterized AP53/covR+S– and AP53/covR+S+ strains were also used for comparison. Flow cytometry and Western blotting techniques were used to determine the interaction of M23ND strains with the complement system. Mouse survival studies were performed in a plasminogen humanized mouse model. Phagocytic assays were conducted with isolated human neutrophils.

Results: Unlike AP53/covR+S–, M23ND/covR+S– cells displayed negligible binding of host complement inhibitors of C3 convertase, viz., Factor H (FH) and C4-binding protein (C4BP). However, despite minimal binding of FH and C4BP, M23ND/covR+S– still showed minimal C3b deposition, weak phagocytosis by neutrophils, and did not protect mice against lethality. Further, M23ND/covR+S– resulted in reduced adherence and internalization by HaCaT cells during adhesion assays.

Conclusion: Our data support the hypothesis that the enhanced virulence in M23ND is due to the unique genetic variation in the CovRS system, which influences bacteria-host interactions and opsonization process.

Disclosure of Interest: None declared.

PO306-TUE

CD44 antibodies can directly inhibit murine macrophage FC-gamma receptor and complement receptor 3 – mediated phagocytosis

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Background: Phagocytosis is thought to play a significant role in the pathobiology of autoimmunity including cytopenias. CD44 antibodies are able to ameliorate autoimmunity and we have thus examined the ability of CD44 antibodies to directly inhibit Fc-gamma receptor (FcγR) – and complement receptor-3 (CR3)-mediated phagocytosis *in vitro*.

Aims: To evaluate the effect of anti-CD44 on FcγRs and CR3 – mediated phagocytosis.

Methods: Peritoneal macrophages (PerM), bone marrow-derived macrophages (BMDM), and the RAW264.7 cell line were evaluated. Macrophages were treated with the CD44 antibody IM7, deglycosylated IM7, or control antibodies and phagocytosis of fluorescent-labeled and IgG-sensitized sheep RBCs (SRBCs) was assessed by confocal microscopy and flow cytometry. for CR3-mediated phagocytosis, macrophages were activated with phorbol 12-myristate 13-acetate and SRBC sensitized with IgM and complement C5-deficient human serum.

Results: CD44 antibody inhibited FcγR-mediated phagocytosis. This inhibition was at the level of binding of the sensitized SRBCs to the macrophages. The inhibitory effect of anti-CD44 was absent in CD44^{-/-}BMDM confirming that CD44 expression is required for this effect. Deglycosylated CD44 antibody which does not bind well to FcγR was also able to inhibit phagocytosis, suggesting that inhibition was not dependent on anti-CD44 – FcγR-interactions. CD44 antibody also inhibited CR3-mediated phagocytosis, demonstrating that inhibition is at a level common for these two distinct mechanisms of phagocytosis.

Conclusion: CD44 antibodies inhibit FcγR- and CR3-mediated phagocytosis. This effect is not likely mediated by the direct binding and blockage of FcγRs. Further study is needed to understand the mechanism underlying this effect and its usefulness for the amelioration of autoimmune cytopenias.

Disclosure of Interest: None declared.

PO307-TUE

Exogenous and endogenous toll-like receptor ligands induce the formation of platelet-neutrophil and platelet-monocyte complexes in human whole blood

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Background: Toll-like receptors (TLRs) recognize their natural pathogen agonists as well as endogenous ligands like histones, which are released under inflammatory conditions. TLR activation results in activation of immune cells and thereby can lead to formation of platelet-neutrophil and platelet-monocyte complexes (PNCs and PMCs). Complex formation facilitates extravasation of the cells into tissues and enhances processes as atherosclerosis and myocardial infarction.

Aims: We tested the effect of various agonist stimulation of human TLRs on PNC and PMC formation.

Methods: Whole blood from healthy donors was analyzed by flow cytometry. PNC levels were quantified after *ex vivo* stimulation with agonists for TLRs 1 to 9, histones H3 and H4. Vehicle and ADP (2 μM, 20 μM) were used as controls. A four color staining was applied with CD45 for leukocytes, CD15 for neutrophils, CD14 for monocytes and CD41 for platelets. PNCs and PMCs were measured as percentage of CD41 positive events of all CD15 or CD14 positive events.

Results: Stimulation with ADP (2 μM, 20 μM) compared to vehicle increased PNC levels by 18% and 57%, respectively ($P < 0.01$), as well as PMC levels by 66% and 56%, respectively ($P < 0.01$). H3 and H4 increased PNC formation by 58% and 64% ($P < 0.01$) whereas TLR stimulation with various agonists did not alter PNC levels significantly. Treatment with H3 produced 60%, with H4 43% more PMCs compared to vehicle ($P < 0.01$). Stimulation with TLR8 agonist increased PMC count by 30% ($P = 0.04$), with TLR4 agonist by 28% ($P = 0.046$).

Conclusion: Histones H3 and H4 as endogenous TLR ligands increase PNC and PMC formation *in vitro* indicating possible immunomodulatory function.

Disclosure of Interest: None declared.

PO308-TUE

Complement activation and thrombin generation in healthy individuals subjected to mild isobaric hypoxia and venous stasis

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Background: A local milieu with severe hypoxia and stasis in the valvular pockets of large veins is the most frequent site for initiation of venous thromboembolism (VTE). The underlying mechanism(s) are unclear, but may involve a cross-talk between complement- and subsequent coagulation activation. Previous studies have reported endogenous thrombin generation secondary to mild hypobaric hypoxia which may explain the modest VTE risk after air flights.

Aims: To investigate whether mild isobaric hypoxia with subsequent venous stasis activates the complement- and coagulation systems

Methods: A randomized, single-blinded study was performed in 30 healthy subjects of which 20 individuals were subjected to air with 16% O₂ and 10 controls to 21% O₂ for 60 min, followed by venous stasis for 20 min. of the upper right arm (cuff with 40 mmHg). Blood was collected from a cannula in the antecubital vein of the right arm. Complement activation was monitored by measurement of activation products by enzyme-immunoassays, whereas endogenous thrombin generation was assessed by TAT and F 1 + 2. The study was approved by the research ethics committee and all subjects gave informed written consent.

Results: Exposure to mild isobaric hypoxia for 60 min was accompanied by a drop in O₂-saturation from 98% to 93% ($P < 0.001$), activation of the complement system as measured by the activation products C4bc (from 3.6 to 5.7 Units mL⁻¹ $P < 0.01$) and C3bBbP (from 11.0 to 16.0 Units mL⁻¹ $P < 0.01$), and endogenous thrombin generation (TAT from 3.0 to 15.4 μg L⁻¹, $P < 0.001$). Except for C4bc showing a trend ($P = 0.08$). Independent of air O₂, subsequent venous stasis was associated with additional increase in thrombin generation, assessed by TAT and F 1 + 2 ($P < 0.05$), but not in complement activation.

Conclusion: Our findings imply that mild isobaric hypoxia is associated with limited activation of the complement- and coagulation systems not statistically different from measurements under normal oxygen supply.

Disclosure of Interest: None declared.

Management of bleeding I

PO309-TUE

Retrospective evaluation of the use of recombinant factor VIIa before and after the implementation of a pharmacist-led factor stewardship program

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Background: Recombinant Factor VIIa (rFVIIa) is FDA approved for use in patients with hemophilia and factor VII deficiency. Additional off-label indications are supported in the literature; however, there are concerns about overuse of rFVIIa in the hospital setting due to high cost and risk of VTE.

Aims: The purpose of this study was to assess how the implementation of a pharmacist-led factor stewardship program at an academic medical center influenced prescribing of rFVIIa in accordance with evidence based institutional guidelines.

Methods: The study was a single center, retrospective quality improvement project. All patients 18 years and older who received rFVIIa between July 2008 and June 2014 were included. Patients were excluded if they had Factor VII deficiency, acquired factor VIII inhibitor, or underwent cardiothoracic surgery or neurosurgery. The primary objective was to compare appropriate rFVIIa prescribing before and after implementation of the factor stewardship program. Appropriate prescribing was defined as dosing and indications that complied with institutional guidelines for factor use. Secondary endpoints included number of doses and mean dose given, amount of blood products given within 24 h pre- and post- dose, hospital length of stay, 24 h and 30 day mortality, and incidence of VTE.

Results: A total of 207 patients were included: 117 patients pre-and 90 patients post-implementation of the factor stewardship program. The mean number of rFVIIa doses decreased from 2.3 to 1.3 and the mean dose decreased from 72.6 to 50.4 $\mu\text{g kg}^{-1}$. Appropriate prescribing increased from 37% to 57%. Mortality rates at 24 h and 30 days were reduced from 23% to 16% and from 47% to 39% respectively.

Conclusion: A pharmacist-led factor stewardship program successfully reduced the use and doses of rFVIIa. At the same time, adherence to institutional prescribing guidelines and mortality rates improved.

Disclosure of Interest: None declared.

PO310-TUE

Use of a 4 factor prothrombin complex concentrate in a United States community hospital system

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Background: A 4 factor Prothrombin Complex Concentrate [Human] (4FPCC) was approved by the United States of America (USA) Food and Drug Administration (FDA) in 2013 for the urgent reversal of vitamin K antagonist therapy in adult patients with acute major bleeding, or the need for an urgent surgery or invasive procedure. To determine if the product was being used appropriately at three hospitals in Albuquerque, New Mexico USA, an audit was performed via retrospective chart review. The study complies with the Declaration of Helsinki.

Aims: The audit was preformed to determine if usage of 4FPCC in three hospitals in a metropolitan area of the USA was in accordance to labeling by the FDA.

Methods: Adult patients who had received 4FPCC in the 6-month time frame for the study were included (April – October of 2014). Patients were identified via the dispensing logs located in the pharmacy department and the electronic medical record (EMR) in a healthcare system that includes three nonprofit community hospitals in Albuquerque, New Mexico USA. 4FPCC use was audited for indication, dosage, the patient's initial International Normalized Ratio value, patient weight, and concomitant vitamin K administration.

Results: Twenty-five patients met the inclusion criteria. All had an FDA-approved indication for 4FPCC administration. All patients received correct dosing as per the FDA labeling however only 19 patients correctly received concomitant vitamin K administration.

Conclusion: 4FPCC was administered to adult patients at our facilities for FDA-approved indications and at the correct dosage for all patients in the study period. However, vitamin K administration, required as adjunctive therapy in the FDA labeling, was not given 24% of the time. This represents an opportunity for improvement at our facilities. Outcomes will be compared for any differences in the group that did not receive vitamin K. Adjustments to the order panel in the EMR may be considered.

Disclosure of Interest: None declared.

PO311-TUE

In vitro optimization of an innovative biological glue composition

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Background: A biological glue with an innovative formulation was proposed using fibrinogen and activated factor VII (FVIIa), as the clotting inducer. The deposition of such a mixture in a wound will allow the contact of FVIIa with endogenous tissue-factor and traces of blood to ultimately clot fibrinogen.

Aims: In preliminary experiments we sought to evaluate the ability of such mixture to clot *in vitro*.

Methods: To establish the mixture and to mimic the *in vivo* mechanism of action, traces of plasma, exogenous tissue-factor, calcium and phospholipids were added *in vitro* to induce the clotting in an appropriate buffer. The clotting efficiency was assessed by one-stage clotting assay, visual detection and thromboelastometry.

Results: The minimum amount required for each of the inducers was first determined. At least 3% of plasma, 0.5 pM of tissue factor, 2.5 mM of calcium and 2 μM of phospholipids were required to transform the glue solution. Then, the concentrations of FVIIa and fibrinogen were let to vary. A ratio of efficiency was established dividing the ROTEM parameter MCF by the total clotting time (CT+CFT). Optimal clotting conditions as well as concentrations limiting the efficacy were determined.

We then aimed to increase the viscosity of the solution to favor its contact with the targeted tissue. A cellulose derivate (Klucel MF 1%, Ashland) was found to be suitable. In its presence, the viscosity was increased 3 times and the solution kept its original clotting parameters (time to clot and efficiency of clotting). In addition, the solution containing fibrinogen, calcium and FVIIa, with or without the gelling agent, kept up to 70% of its activity following a 7 days incubation at 25 °C.

Conclusion: This study allowed generating an efficient glue mixture with an increased viscosity possessing a long-lasting hemostatic effect. This property that differentiates this glue from the classical biological glues may confer a significant advantage *in vivo* in controlling during time the blood loss from the wound.

Disclosure of Interest: J.-L. Plantier Employee of: LFB Biotechnologies, V. Duretz Employee of: LFB Biotechnologies, S. Chtourou Employee of: LFB Biotechnologies, P. Mondon Employee of: LFB Biotechnologies.

PO312-TUE

4F-PCC (Beriplex® P/N) is superior to 3F-PCC for reversal of coumarin anticoagulation in a rat bleeding model

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Background: Four factor prothrombin complex concentrate (4F-PCC), containing the human coagulation factors II, VII, IX and X, but not 3F-PCC (containing only minimal VII) is indicated for the urgent reversal of acquired coagulation factor deficiency induced by VKA therapy. Nevertheless, the use of 3F-PCC has been discussed for correcting hemostasis following warfarin overdose.

Aims: The study was conducted as a head-to-head comparison of a 4F-PCC and three factor PCCs (3F-PCC) for effective reversal of vitamin K antagonist (VKA) induced anticoagulation.

Methods: Rats received 2.5 mg kg⁻¹ phenprocoumon p.o. At 15.75 h post dosing, animals were treated with a single i.v. dose of saline, 4F-PCC (Beriplex® P/N, Kcentra®, CSL Behring) or 3F-PCC (Bebulin® VH, Baxter; Profilnine® SD, Grifols). Study endpoints included bleeding following tail clip, activated partial thromboplastin time (aPTT), prothrombin time (PT) and determination of plasma levels of vitamin K dependent coagulation factors.

Results: Coumarin anticoagulation of rats induced a rise in median bleeding time by ≥2 fold from an average of 823 to 1800 s (max. observation period). In parallel, PT and aPTT were prolonged from 8.9 to 29.9 s and 14.5 to 25.5 s, respectively. Treatment with 4F-PCC was able to fully and statistically significantly reverse bleeding, achieving average bleeding times of 676 s. The two 3F-PCCs were not or only partially able to reduce coumarin induced bleeding with average bleeding times of 1398 and 1708 s post treatment, respectively. PT could only be normalized by 4F-PCC but not by 3F-PCCs. There was no reduction in aPTT seen for any treatment option.

Conclusion: This first direct comparison of 4F-PCC and 3F-PCCs for the reversal of VKA anticoagulation in a rat model of acute bleeding suggests that replenishment of all four vitamin K-dependent coagulation factors including factor VII as achieved using a 4F-PCC may result in superior efficacy compared to the use of 3F-PCCs.

Disclosure of Interest: E. Herzog Employee of: Employee of CSL Behring GmbH, F. Kaspereit Employee of: Employee of CSL Behring GmbH, W. Kregge Employee of: Employee of CSL Behring GmbH, P. Niebl Employee of: Employee of CSL Behring GmbH, G. Dickneite Employee of: Employee of CSL Behring GmbH.

PO313-TUE

Evaluation of the performance of eight scores for prediction of bleeding risk in Brazilian heart disease outpatients treated with warfarin

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Background: Warfarin has been used successfully to reduce thromboembolism risk. However, bleeding complications are common. Several

prediction models were developed to estimate the risk of bleeding during anticoagulation therapy. However, there was no validation of these models in Brazilian population.

Aims: The aim of the study is to compare the performance of eight prediction models for bleeding risk in warfarin-treated outpatients.

Methods: The dataset derived from a clinical trial conducted to evaluate the efficacy of an anticoagulation clinic at university hospital in Brazil. A total of 280 outpatients with heart diseases and chronic indication to use warfarin were enrolled. Eight prediction models (OBRI, Kuijer et al., Kearon et al., HEMORR₂HAGES, Shireman et al., RIETE, HAS-BLED and ATRIA) were compared to evaluate the overall model performance by Nagelkerke's R² estimation, discriminative ability by the concordance (c) statistic and calibration of the models by the Hosmer-Lemeshow goodness-of-fit statistic. The primary outcome variables were the first episodes of major and minor bleeding events within 12 months of follow-up.

Results: Major bleeding occurred in 14 participants (5%), minor bleeding in 159 (56.8%) and no bleeding at all in 119 (42.5%). The majority of the participants who experienced major bleeding had their risk misclassified. All the models presented poor discriminative ability (c index ranged from 0.423 to 0.545) and low overall performance (Nagelkerke's R² values ranged from 0.0% to 0.4% for minor bleeding and from 0.0% to 2.2% for major bleeding prediction). Only OBRI, Kearon et al., HEMORR₂HAGES and RIETE models presented good calibration for predicting both major and minor bleedings.

Conclusion: All the models tested demonstrated poor performance for predicting minor and major bleeding events in Brazilian patients, suggesting that more accurate models for this population should be investigated.

Disclosure of Interest: None declared.

PO314-TUE

Idarucizumab specifically reverses dabigatran anticoagulation but does not induce a procoagulant state in a porcine trauma model with dabigatran anticoagulation

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Background: Idarucizumab is an antibody fragment (Fab) designed to inhibit the anticoagulant effect of dabigatran. Ideally, reversal agents should only antagonize or neutralize the anticoagulant without inducing a procoagulant state.

Aims: This study assessed any potential procoagulant properties of idarucizumab in a porcine polytrauma model with dabigatran anticoagulation.

Methods: Oral dabigatran etexilate (30 mg kg⁻¹ bid, n = 12) or placebo (sham, n = 6) was administered to male pigs for 3 days and infused on day 4 for consistent dabigatran levels. All animals underwent blunt liver injury and were randomized to idarucizumab, 120 mg kg⁻¹ (Fab 120) or saline (dabigatran control) 15 min post injury. Blood loss (BL), thrombin generation (TG) and platelet aggregation (PA) were tested for 4 h or until death. Dabigatran levels were measured as dTT (Hemoclot) and TG with thrombinography (5 pM tissue factor, TF). PA to TF, 1:2000 dilution, collagen (2 µg mL⁻¹) or adenosine diphosphate (5 µM ADP) was measured using light transmission aggregometry for 5 min and assessed as AUC (V*s).

Results: Dabigatran levels were 1205 ± 414 ng mL⁻¹ prior to trauma. This level of anticoagulation resulted in complete inhibition of TG (ETP 0 ± 0 nM*min). After dosing Fab 120, ETP increased to 393 ± 47 nM*min, which was comparable to TG in sham animals (ETP: 346 ± 46 nM*min). Bleeding stopped within 15- 30 min (total BL 1140 ± 109) and dabigatran levels immediately decreased to 0 ng mL⁻¹. TG in controls was not measurable and total BL increased

to 2977 ± 316 mL. TF-induced PA was inhibited from 226 ± 32 V*s in sham group to 10 ± 16 V*s in controls ($P < 0.001$). Aggregation normalized after Fab 120 to 197 ± 27 V*s at 120 min ($P > 0.05$ vs. sham). Collagen and ADP-induced aggregation were not affected.

Conclusion: In this lethal porcine trauma model, idarucizumab significantly decreased blood loss induced by supratherapeutic dabigatran and blunt liver injury. This was associated with a return of anticoagulation to baseline without inducing a hypercoagulable state.

Disclosure of Interest: M. Honickel: None declared, J. van Ryn Employee of: Boehringer Ingelheim, J. Schurer Employee of: Boehringer Ingelheim, R. Rossaint Consultant for: CSL Behring, Novo Nordisk, O. Grottko Grant/Research Support from: CSL Behring, Novo Nordisk, Boehringer Ingelheim, Consultant for: Bayer Healthcare, Portola, Boehringer Ingelheim.

PO315-TUE

Whole blood thromboelastometry profiles in patients undergoing major orthopaedic surgery

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Background: Major orthopaedic surgery is characterized by thrombotic and haemorrhagic complications. Several studies have failed to demonstrate the usefulness of classical coagulation parameters to predict both the thrombotic and the bleeding risk. Using whole blood for coagulation analysis theoretically could be more favourable compared with plasma. Whole blood rotation thromboelastometry (ROTEM®, Tem International GmbH, Munich, Germany) is a point-of-care global coagulation analyzer able to evaluate the characteristics of clot formation and lysis by dynamic monitoring.

Aims: To record thromboelastographic profiles, using ROTEM®, in patients undergoing major orthopaedic surgery.

Methods: Twenty patients (M/F 8/12, age 48–90) undergoing major orthopaedic surgery were consecutively enrolled. Samples were collected immediately pre-surgery (T0-pre), immediately post conclusion of the surgery (T0-post), 1 day post the surgery (T1) and 5 ± 2 days post surgery (T2). Intrinsic (INTEM) and extrinsic (EXTEM) coagulation pathways and the function of fibrinogen (FIBTEM) were evaluated. Thromboelastographic parameters considered were clotting time (CT sec.) and clot formation time (CFT sec.) in INTEM and EXTEM and maximum clot firmness (MCF mm) in FIBTEM.

Results: CT in INTEM and EXTEM was similar at each time-points considered in the study. CFT (mean ±SD) in INTEM and EXTEM were significantly prolonged at T0-post (71.6 ± 35.0 and 82.35 ± 52.3 s, respectively) than at T0-pre (57.6 ± 20.0 and 59.1 ± 14.4 s, respectively; $P < 0.01$ in both cases). MCF in FIBTEM was significantly lower at T0-post than at T0-pre (19.0 ± 9.0 v 24.2 ± 11.7 mm, respectively; $P < 0.01$)

Conclusion: Thromboelastometry, performed by ROTEM® immediately after surgery identified a hypocoagulable profile. These changes may contribute to the bleeding risk that occurs in the perioperative period. Future prospective studies aimed at correlating hemostatic changes with perioperative outcomes are warranted.

Disclosure of Interest: None declared.

PO317-TUE

A multicenter observational study of the effectiveness of prothrombin complex concentrate for treatment of bleeding in the perioperative and intensive care setting: probe study

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Background: Few studies have investigated the use of prothrombin complex concentrates (PCCs) to control bleeding unrelated to oral anticoagulation.

Aims: To examine real-life use of PCC in perioperative and intensive care settings in the UK.

Methods: This observational, non-interventional study was conducted across nine centers. Patients with coagulopathy who required surgical/diagnostic intervention and prophylactic hemostatic treatment (cohort P), or with acute perioperative bleeding requiring hemostatic treatment (cohort T) were included. Patients treated with oral vitamin K antagonists were excluded. The study was approved by an independent ethics committee and informed consent was waived for the data collection.

Results: 372 patients received PCC (cohort P: 21; cohort T: 351; PCC alone: 244) and 73 received fresh frozen plasma (FFP) only (cohort P: 19; cohort T: 54). In cohort P, PCC/FFP was administered prophylactically for a wide variety of surgical/medical procedures and investigations. Cardiac surgery was the most common setting in cohort T. The median total dose of PCC in cohort P was 22.5 (IQR: 18.1–27.3) IU kg⁻¹, compared with 11.5 (IQR: 7.1–15.4) IU kg⁻¹ in cohort T. Clinical effectiveness was judged as very good or satisfactory in 93.0% of patients receiving PCC alone (cohort P: 87.5%; cohort T: 93.4%). There was no significant association between dose and effectiveness among recipients of PCC alone, though bleeding severity differences may have confounded this analysis. Seven thromboembolic events were considered as possibly related to PCC, an incidence comparable to observational studies in cardiac surgery without PCC. One death in a 70-year-old woman with acute promyelocytic leukemia was possibly related to PCC treatment.

Conclusion: This study shows the potential for using PCC to treat coagulopathic bleeding in clinical settings beyond oral anticoagulation reversal. The treatment was well tolerated and may have contributed to the effective management of bleeding.

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PO318-TUE

Bleeding complications after invasive dental procedures in patients under vitamin K antagonists

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Background: Increasing evidence in literature report that invasive dental procedures (IDP) can be safely performed within therapeutic international normalized ratio (INR), in patients on vitamin K antagonists (VKA). However bleeding still occur due to its inherent risk.

Aims: To evaluate the prevalence of hemorrhages after IDP in patients under VKA, with INR $\leq$ 4 on procedure's day.

Methods: Detailed IDP was assessed by a questionnaire. Bleeding complications were self-reported if no medical intervention was required, otherwise clinical registries were reviewed.

Results: Overall, 333 IDP were evaluated, corresponding to 179 patients. 32.4% of them were anticoagulated due to atrial fibrillation. IDP were: 188 tooth extraction, 127 scalping, 14 dentistry and 4 root canal therapy. Prevalence of hemorrhage was 10.2% ($n = 34$), the majority ($n = 32$) classified as minor local hemorrhage solved spontaneously or with local hemostatic measures using antifibrinolytic, within 24 h. Only two patients had moderate bleeding and attended the emergency service, both after one tooth extraction with therapeutic INRs (2.87, 2.1). Reversion of VKA was performed with vitamin K and prothrombin complex concentrate. No transfusions or hospitalization were required.

Considering the type of IDP, bleedings occurred in 16.5% ($n = 31$) of the teeth extractions and in 2.4% ($n = 3$) of the scalping. Bleeding after extractions were unrelated to the teeth number extracted: 11.7% ($n = 22$) with one tooth extraction, 4.3% ($n = 8$) with two and 0.5% ($n = 1$) with three teeth extracted. Post-IDP hemorrhage was unrelated to INR, as 23% ($n = 8$) of the patients had INR $<$ 2.0, 55% ($n = 19$) $\geq$ 2.0– $\leq$ 3.0 and 20.6% ($n = 7$) $>$ 3.0– $\leq$ 4.0.

Conclusion: Tooth extraction was the intervention with more minor bleeding episodes. Moderate hemorrhages were rare and severe complications were not seen. In conclusion, an adequate hemostasis can be obtained in most situations using appropriate local hemostatic measures in patients with therapeutic INR.

Disclosure of Interest: None declared.

PO319-TUE

An analysis of management strategies and outcomes in NVAF patients treated with dabigatran: a multi-center retrospective cohort of patients with major bleeding events

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Background: The new oral anticoagulant dabigatran etexilate (DE) 150 mg carries a similar risk of major hemorrhage as warfarin. A

recent meta-analysis concluded that the outcome of major bleeding events in 5 phase III trials was no worse in DE-treated patients than in warfarin-treated patients. However, the management of major bleeding events in non-study patients on dabigatran has yet to be studied systematically.

Aims: We performed a structured multi-center analysis of electronic medical record (EMR) data of non-valvular atrial fibrillation (NVAF) patients who presented with major bleeding events while being treated with DE at 5 academic centers to evaluate management and outcomes in routine clinical care.

Methods: EMR databases were queried for NVAF patients presenting to the emergency department with ICD-9 codes for bleeding events, and who were treated with DE. These charts were then systematically reviewed by site staff to identify patients who met the International Society of Thrombosis and Hemostasis (ISTH) definition of major bleeding and to confirm the patient had taken at least one dose of DE within 5 days prior to the index event. Exclusion criteria included documentation that the patient was taking a concomitant anticoagulant, was receiving thrombolytic therapy or enrolled in an interventional investigational study at the time of the onset of the index event. The final cohort was examined for demographic data, types of bleeds, treatments provided and outcomes.

Results: Results including demographic data, types of bleeds, treatments provided for management of the events and outcomes will be reported.

Conclusion: To be provided upon review of final results.

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PO320-TUE

Use of prothrombin complex concentrate and activated prothrombin complex concentrate for reversal of direct oral anticoagulant associated bleeding

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Background: The management of life threatening bleeding associated with the direct oral anticoagulants (DOACs) is controversial. Previously published small studies have shown that prothrombin complex concentrate (PCC) and activated PCC (aPCC) therapies may be safe and effective to control these bleeding episodes.

Aims: To describe the clinical outcomes of DOAC-associated life threatening bleeding events managed using PCC and aPCC.

Methods: Retrospective review of patients presenting with life threatening bleeding events between January 2013 and June 2014 and were managed using PCC or aPCC. The primary outcome was occurrence of venous and arterial thrombotic events during hospitalization. The secondary outcome was symptomatic control of bleeding.

Results: Twenty-one patients presented with life threatening bleeding events. Ninety percent of the patients were anticoagulated for atrial fibrillation, 10 were on rivaroxaban. Twelve patients received PCC (57%; median dose: 1750 IU; range: 1000–2500 IU) and 9 received aPCC (43%; 3000 IU; 1740–6111 IU). All patients received supportive care and interventions to attain source control when possible. Patients treated with PCC were more likely to be transfused additional hemostatic blood products with 50% getting rFVIIa vs. 0% in aPCC group. Adverse events after receiving PCC and aPCC were uncommon. One patient experienced a TIA post-aPCC and deficits resolved by hospital

discharge. Two patients in each group had evidence of ongoing bleeding. Three patients died of major bleeding (one in PCC group).

Conclusion: DOAC-associated life threatening bleeding episodes managed using PCC or aPCC seems to be associated with a low rate of adverse events. These therapies appear to be helpful in controlling bleeding episodes, when combined with source control. Prospective studies are needed to evaluate benefits and harms of PCC and aPCC for management of DOAC-associated life threatening bleeding episodes.

Disclosure of Interest: None declared.

PO321-TUE

Treatment approaches in spontaneous rectus sheath hematomas in clinical practice. a single-center experience

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Background: Spontaneous rectus sheath hematoma (RSH) is a relatively rare condition that is usually associated with antithrombotic treatment and whose management is not well established.

Aims: To investigate the clinical presentation and treatment of RSH patients referred to the Hemostasis Unit in a tertiary hospital.

Methods: A retrospective analysis was performed on all RSH patients treated from January 2008 to May 2014.

Results: A total of 32 patients were identified during the review period. Twenty-three cases (69%) were females and the median age at diagnosis was 73 years (51–86). All patients were anticoagulated, 68% with acenocoumarol and 31% with low molecular weight heparin (LMWH). Fifty-nine percentage of subjects were classified as high thrombotic risk (42% prosthetic mechanic heart valves). According to HASBLED/RIETE bleeding score, half of them (53%) were included in the higher group. The median renal clearance was 58 ml min⁻¹ (23–120) and 7 patients (21%) had prior bleeding history. Nearly half of the events (46%) were coughing fit related. The most common clinical presentation was a palpable mass associated with abdominal pain. In 12 patients (37%), doses of antithrombotic therapy were supratherapeutic. Reversion strategies (36%) were diverse and included vitamin K, fresh frozen plasma, prothrombin concentrate and protamine sulfate. While a conservative approach was elected in 20 cases (59%), 12 (37%) patients underwent endovascular embolization successfully. The hospitalization average was 14 days and 12% died, usually related to other causes. At discharge, 56% of patients were treated with at least intermediate doses of LMWH. The median time to reinstitute oral anticoagulation (OA) was 28 days (1–120).

Conclusion: In our cohort RSH occurs mostly in anticoagulated patients with high thrombotic and bleeding risk. Although treatment options were heterogeneous, vascular embolization was only required in one-third of the cases and resumption of OA was usually postponed after discharge.

Disclosure of Interest: None declared.

Microparticles II

PO322-TUE

Synthesis and *in vitro* studies of nanoxides as hemostatic agents for the blood coagulation acceleration

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Background: Hemorrhage is one of the main cause of trauma related deaths. The development of an effective method to treat uncontrolled bleeding has become a priority. Studies involving the use of zeolites as hemostatic agents have shown encouraging results for the control of hemorrhage. When in contact with the blood, zeolites can adsorb the blood water molecules, therefore concentrating the proteins and cellular elements to speed the clot formation.

Aims: This study reports the synthesis, characterization and *in vitro* hemostatic efficacy of the as made nanometric faujasite zeolite and its calcium ion exchange derivate. The interaction of the zeolite surface and the blood proteins can be understood through the *glass effect* mechanism. The main parameters obtained by Thromboelastograph are: R, K (times to reaching a 2 mm and 20 mm clots respectively) α angle and MA (conversion of fibrinogen to fibrin and maximum amplitude respectively).

Methods: The ion exchange experiments were performed with aqueous salt solutions under controlled conditions. The materials were characterized by DRX, MEV and TEG was employed to assay the *in vitro* hemostatic efficacy.

Results: Experimental results of MEV and DRX were in agreement with the pattern FAU zeolite reported in literature. Studies using TEG analysis showed that the parameters such as time R, K, α angle and MA suffer a modification. The time R was reduced by 6.6 min and the time K reduced by 2.9 min when the FAU as made is in blood. Blood with ion-exchange material reduced by 7.6 min in R and 2.8 min in K. Besides the reduction of time to clot formation, there was also an increase in thrombin generation and increased platelet adhesion.

Conclusion: Experimental TEG parameters have showed that the blood clots more quickly when in contact with FAU nanoxide. Calcium ion exchange derivatives were more efficient in comparison with the as made nanozeolite. The results have indicates that zeolites materials are hemostatically active and capable of efficiently promoting blood-clot formation.

Disclosure of Interest: None declared.

PO323-TUE

Composition-dependent effects of nanoparticles on coagulationBakhtiari K^{1,2}, Meijers JC^{1,2}, Laurent S³, Mintova S⁴, Ng E-P⁵, Awala H⁴, Mirsadeghi S⁶, Mahmoudi M^{6,7} and Rezaee F^{8,9}¹Department Plasma Proteins, Sanquin Research; ²Department of Experimental Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands;³Department of General, Organic, and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, University of Mons, Mons, Belgium; ⁴Laboratory of Catalysis and Spectroscopy, ENSICAEN, University of Caen, Caen, France; ⁵School of Chemical Sciences, Universiti Sains Malaysia, Penang, Malaysia; ⁶Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran; ⁷Division of Pediatric Cardiology, Department of Pediatrics, Stanford University School of Medicine, Stanford, USA; ⁸Department of Gastroenterology and Hepatology, Erasmus Medical Center, Rotterdam; ⁹Department of Cell Biology, clinical proteomics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands**Background:** With a rapid increase in nanoparticle (NP) applications in biomedicine, the synthesis of many categories of NPs increases too. The potential risk of these NPs remains unknown. One of the potential affected pathways is blood coagulation.**Aims:** To investigate the effects of four categories of NPs: 1-superparamagnetic iron oxide nanoparticles (SPIONs) that were uncoated or coated with N- or C-dextran, 2-Gold (Gold sphere 20 nm, Gold sphere 30 nm, Gold-short rod and Gold-Long rod), 3-Nano-sized zeolite and 4-Silica, on coagulation using hemostatic assays.**Methods:** Diluted prothrombin time, calibrated automated thrombography (CAT) and rotation thromboelastometry (ROTEM) in plasma and whole blood were used to assess the effect of NPs on coagulation. Effects of NPs on thrombin activity were determined in a purified. The thrombin time was used to assess effects of NPs on fibrin polymerization.**Results:** In plasma, the N-dextran coated SPIONs and Gold NPs showed concentration-dependent prolongation of tissue factor (TF)-initiated coagulation, while uncoated and C-dextran coated SPIONs showed stimulation at low but inhibition at higher NP concentrations. Nano-zeolite NPs accelerated tissue factor-initiated coagulation, while silica NPs had no effect. In whole blood, the results with TF-initiated coagulation matched those of the plasma system, except for uncoated SPIONs, which had no effect on coagulation at higher doses. Uncoated SPIONs acted via disruption of fibrin polymerization, while C-dextran coated SPIONs directly inhibited the active site of thrombin. The effect of N-dextran coated SPIONs and one of the Gold (short rod) NPs appeared to be mediated by platelets. Silica and three other Gold NPs did not influence TF-initiated coagulation.**Conclusion:** NPs differentially affect the coagulation process depending on their chemical composition. The interaction with coagulation should be taken into account when designing NPs for future applications in biomedicine.**Disclosure of Interest:** None declared.

PO324-TUE

Platelet activation releases mature MIR-494 in microparticlesTay J¹, Jorritsma J¹, Hughes Q¹ and Baker RI^{2,3}¹WA Centre for Thrombosis and Haemostasis, Murdoch University, Murdoch; ²Perth Blood Institute, Nedlands; ³WACHT, Murdoch University, Murdoch, Australia**Background:** Platelets are major contributors of circulating miRNA pools and contain Dicer and Argonaute-2 complexes that process pre-miRNA transcripts into mature miRNA. We identified an oestrogen-dependent miR-494 expression profile, where inhibition of *PROS1* expression by miR-494 is an indirect mechanism for acquired Protein S (PS) deficiency in pregnancy. We have investigated whether miR-494 expression is detectable in human plasma and if platelet activation leads to increased processing of pre-miR-494 and mature miR-494 release in platelet microparticles (PMPs).**Aims:** Determine if miR-494 is detected in plasma, associated with PMP and if platelet activation increases mature miR-494 levels.**Methods:** Citrated venous blood was collected from nonpregnant females and platelet rich plasma (PRP) was stimulated with 1 mg mL⁻¹ collagen for 60 min and compared to unstimulated control PRP, double spun platelet poor plasma (PPP) and ultracentrifuged PPP (20,000 g × 90 min). Samples were stained and analysed by flow cytometry for platelet activation (GPIIIa/P-Selectin) and PMP quantitation (Annexin-V/PECAM-1/GPIIIa/IIIb complex). Total RNA was extracted from plasma samples, and the level of mature miR-494 was determined by digital droplet PCR (DDPCR) and RT-qPCR.**Results:** In PPP, miR-494 is detectable by DDPCR and RT-qPCR, and not detectable in ultracentrifuged samples. Collagen-stimulated PRP samples demonstrated platelet activation by increased in P-Selectin expression and a corresponding 2-fold increase in PMPs compared to unstimulated samples. Levels of miR-494 were increased by approximately 60% following platelet activation.**Conclusion:** Platelet activation increases PMP generation and miR-494 expression, suggesting that higher circulating PMP levels contain increased levels of mature miR-494. PMPs may be the mechanism for the delivery and control mechanism for the fall in PS levels with high oestrogen levels with pregnancy.**Disclosure of Interest:** None declared.

PO325-TUE

Characteristics and role of extracellular vesicles in the pathophysiology of transfusion dependent β -ThalassemiaLevin C^{1,2,3}, Sabbah A², Brenner B² and Aharon A²¹Pediatric Hematology Unit, Emek Medical Center, Afula;²Thrombosis and Hemostasis Unit and Department of Hematology, Rambam Health Care Campus; ³The Bruce Rappaport Faculty of Medicine, Technion, Haifa, Israel**Background:** Patients with β -Thalassemia Major (TM) require blood transfusion for life and develop severe dysfunctions in major organs and systems; however not all complications can be explained by iron overload.**Aims:** Characterization of extracellular vesicles (EV) in TM and evaluation of their role in the pathogenesis of TM complications.**Methods:** Thirty transfusion dependent β -Thalassemia (TDT) patients and 30 controls (C) were included. TDT patients were divided into 3 groups: 1) nonsplenectomized, 2) splenectomized (SP) and 3) hypersplenic (HS). EV characteristics (cellular origin, cytokine content, thrombogenicity) were assessed by FACS and protein array. Apoptotic effects of patients' EVs on endothelial and liver cells were evaluated (TUNNEL).

Results: EV count μL^{-1} was lower in HS (178 ± 96) compared to C (410 ± 167 , $P = 0.02$) and SP (1812 ± 1937 , $P = 0.001$). The % of RBC-EVs was lower in HS (22.5 ± 20) vs. C (35.4 ± 13 , $P = 0.03$) and SP (34.2 ± 18 , $P = 0.06$) and it correlated with the hematocrit in HS and SP groups. The % of annexin-V labeled and EPCR-labeled EVs were lower in patients (28 ± 13 ; 19.2 ± 14) vs. C (15.1 ± 10 , $P = 0.01$; 32.9 ± 17 , $P = 0.009$) respectively. Substantial differences in cytokines and angiogenic proteins between the controls and patients were found. The amount of 11 proteins in SP-EVs was twice as high compared to that of controls. Patient EVs induced higher apoptotic rate on endothelial and liver cells ($16.5\% \pm 10$; $20.9\% \pm 6.6$) compared to control EVs ($7.6\% \pm 5$, $P = 0.007$; $14.8\% \pm 5$, $P = 0.09$) respectively.

Conclusion: Important differences in EV properties (EV number, exposure of negatively charged phospholipids, cell origin and cytokine/antigenic protein content) were found between patient and control EVs. The lower % of the anti-coagulant EPCR-labeled EVs observed in patients might contribute to the prothrombotic tendency in TM. Patient EVs increased apoptosis in cultured cells, suggesting a novel mechanism of organ damage in TM.

Disclosure of Interest: None declared.

PO326-TUE

Microparticles: what's plasma made of?

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Background: Microparticles (MPs) are proposed to play major roles in health and disease. However, despite intense research, our knowledge on MPs is limited, mainly due to their small size, their heterogeneity and the limitations of characterization methods. There is a critical need for reliable methods enabling the quantification of MPs in health and disease.

Aims: Our objective is to develop a simple and general method for enumerating the main populations of MPs in plasma of healthy subjects, as a first step towards the development of diagnosis assays.

Methods: Ten platelet free plasma (PFP) samples were labeled with Annexin5 (Anx5)-Cy5, anti-CD41-or anti-CD235a-mAb-PE. MP concentrations were determined by flow cytometry (FCM) on a Gallios.

Results: We have developed a simple FCM approach that enables to detect about 50 times more MPs than conventional FCM methods based on light scatter triggering (1). In this approach, MPs are detected on the basis of their fluorescence (FL) intensity. We determined the concentrations of the main MP populations in PFP, originating from platelets or erythrocytes, as well as their sub-populations that bind or not Anx5 (2). We find that PFP contains about 50,000 MPs per μL . About 50% of plasmatic MPs bind Anx5, out of which 30% are of platelet origin and only 3% of erythrocyte origin. The MP concentrations determined by FL triggering are close to those determined by electron microscopy after on-grid sedimentation (3). Finally, we found detection limits of about 1000 marker molecules, suggesting that MPs down to 100–150 nm diameter are detected by FL triggering.

Conclusion: The enumeration of MPs by FL triggering is a simple, reliable and highly sensitive method. The catalogue of MPs in normal plasma constitutes a reference for disease studies. This work opens avenues towards the development of standardized procedures for MP analysis.

1. Arraud et al. J. Thromb. Haemost. DOI: 10.1111/jth.12767.

2. Arraud et al., Cytometry A (in revision).

3. Arraud et al. J. Thromb. Haemost. 2014 12: 614.

Disclosure of Interest: None declared.

PO327-TUE

Coagulation activity of microparticles from different cellular sources. involvement of active tissue factor

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Background: Circulating microparticles (MPs) are formed upon activation and/or apoptosis of different blood cells and endothelial cells (ECs). MPs are procoagulant due to the exposure of phosphatidylserine and the presence of tissue factor (TF) in some of them.

Aims: To compare coagulation activity and TF activity of MPs from platelets, monocytes, granulocytes and ECs.

Methods: MPs were obtained from human blood platelets, monocytes and granulocytes, from cultured monocytic THP-1 cells and human umbilical vein ECs. Platelets were activated by thrombin activating peptide, and other cells by lipopolysaccharide. MPs were sedimented (20000 g, 30 min) from the supernatant or culture fluid of activated cells, resuspended in Tris-saline with 1% albumin, and stored at $+4^\circ\text{C}$ for ≤ 3 days or at -70°C for several months. Coagulation activity of MPs was evaluated by clotting of recalcified human plasma devoid of endogenous MPs (20000 g, 90 min), which was followed by absorbance at 450 nm in microplate wells. TF activity was measured by activation of factor X in the presence of factor VIIa in chromogenic assay.

Results: Plasma clotting was significantly accelerated (decrease of lag phase and increase of maximal rate) after addition of all tested MPs. Maximal coagulation levels (short lag phase and high maximal rate) were registered with MPs from ECs, intermediate – with MPs from platelets, monocytes and THP cells, and minimal – with MPs from granulocytes. Anti-TF antibodies prolonged the lag phase in the presence of MPs from ECs, monocytes and THP cells, but not from platelets and granulocytes. In accordance with these results TF activity (factor VIIa/factor X chromogenic assay) was detected in MPs from ECs, monocytes and THP cells, but not from platelets and granulocytes. Lactadherin (phosphatidylserine blocker) completely abolished coagulation activity of all MPs.

Conclusion: Coagulation activity of MPs from ECs, monocytes and THP cells, but not from platelets and granulocytes was partially depended on the presence of active TF.

Disclosure of Interest: None declared.

PO328-TUE

Impact of various anticoagulants and storage time on plasma concentration and size distribution of extracellular vesicles

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Background: Extracellular vesicles (EVs) sized 50–1000 nm might play an important role in the pathophysiology of many diseases, including venous thromboembolism (VTE). Optimal pre-analytical handling is essential for valid and reproducible measurements of plasma concentration and size distribution of EVs.

Aims: To investigate the impact of 4 different anticoagulants and elapsing time on plasma concentration and size distribution of EVs.

Methods: Blood was drawn from 10 healthy individuals into regular blood collection tubes containing citrate, EDTA, CTAD or heparin as

anticoagulant. EVs were measured using NanoSight NS300 and categorized by size (total count, <100, 100–199, 200–299 and 300–1000 nm). Plasma was either isolated immediately by centrifugation, or the effect of time (1, 2, 4, and 6 h between blood collection and centrifugation) on concentration and size distribution of EVs was studied. Transmission electron microscopy (TEM) was used to confirm the presence of bilayer membranes of EVs. Scanning electron microscopy was used to provide a general overview of the morphology and purity of EVs.

Results: There were no significant differences in total plasma concentrations and size distribution of EVs between anticoagulants when plasma was processed instantaneously. Heparin had higher concentration of large EVs than the other anticoagulants, which accounted only for a small proportion of total EVs. The plasma concentration and size distribution of EVs varied significantly by storage time without any particular trend for all anticoagulants. TEM revealed mainly round-shaped particles with a bilayer phospholipid membranes and equal appearance between anticoagulants. The high purity of EVs did not differ between anticoagulants.

Conclusion: We observed a significant but unpredictable effect on plasma concentration of EVs with elapsed time for all sizes and in all anticoagulants. The type of anticoagulant did not influence concentration or size distribution of EVs when plasma was processed instantaneously.

Disclosure of Interest: None declared.

PO329-TUE

Plasma concentration and size distribution of extracellular vesicles in healthy individuals subjected to mild isobar hypoxia and venous stasis

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Background: Extracellular vesicles (EVs) sized 50–1000 nm might play an important role in the pathogenesis of many diseases, including venous thromboembolism (VTE) due to their procoagulant properties. Patients with exacerbation of chronic obstructive pulmonary disease (COPD), associated with low oxygen saturation and increased vascular resistance in the pulmonary circulation, have elevated plasma levels of EVs and are at high risk of VTE, particularly of pulmonary embolism. However, it is unknown whether the rise in EVs is caused direct by hypoxia or indirect through co-morbid conditions such as acute infections.

Aims: To investigate whether mild isobar hypoxia with subsequent venous stasis increase plasma concentration and alter size distribution of EVs.

Methods: A randomized, single-blinded study was performed in 30 healthy subjects of which 20 individuals were subjected to air with 16% O₂ and 10 subjected to 21% O₂ (controls) for 60 min., followed by venous stasis for 20 min. of the upper right arm (cuff with 40 mmHg). Blood were collected from a cannula in the antecubital vein of the right arm. EVs were measured using NanoSight NS300 and categorized by size (total count (50–1000 nm), <100, 100–199, 200–299 and 300–1000 nm). The study was approved by the research ethics committee and all subjects gave informed written consent.

Results: Exposure to mild isobar hypoxia for 60 min was accompanied by a drop in O₂-saturation from 98% to 93% ($P < 0.001$) without affecting the total plasma concentration of EVs. However, subsequent venous stasis in addition to mild hypoxia caused a moderate increase in total plasma concentration of EVs from $1858 \times 10^7 \text{ mL}^{-1}$ (1733–2375) (median with interquartile range) to $2648 \times 10^7 \text{ mL}^{-1}$ (2348–

3026). The stasis-induced increase in plasma concentrations of EVs did not differ between groups.

Conclusion: Our findings imply that plasma concentrations of EVs are not affected by mild isobar hypoxia, but increased moderately in response to venous stasis.

Disclosure of Interest: None declared.

PO330-TUE

New technique to study cell-derived microparticles

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Background: Microparticles (MPs) are cell-derived vesicles released from cell membranes of a variety of cells in response to activation or apoptosis. They have a phospholipid bilayer which is rich in phosphatidylserine and are in the range from 0.1 to 1 µm. Elevated levels of MPs have been found in vascular, immune and hemostatic disorders and several MP-populations have been shown to be promising biomarkers in cardiovascular diseases. Flow cytometry is a high through-put method allowing enumeration and characterization of MPs. However, the small size and consequently weak signal from most MPs challenge the sensitivity of the flow cytometer. The new generation of flow cytometers have an increased sensitivity and are able to accurately measure MPs down to 0.3 µm (High-sensitivity flow cytometry).

Aims: We are setting up a new technique to study MPs using the solid phase proximity ligation assay (sPLA) and evaluating it by comparison with high-sensitivity flow cytometry.

Methods: An antibody is used to capture MPs in plasma on a solid matrix. Oligo-conjugated antibodies then bind to proteins on the MP-surface. After a ligation step ligating the oligos, the ligation product is detected using quantitative PCR. With this method we can with high sensitivity detect different types of MPs of all sizes.

Results: We have shown a 20-fold increase in the number of tissue factor positive platelet-derived MPs in plasma after stimulation with 20 µM ADP 2 h. A good correlation between results from sPLA and high-sensitivity flow cytometry analysis was found (Pearson R : 0.9869; $P = 0.0131$).

Conclusion: This method shows good potential for sensitive and specific analysis of microparticles as biomarkers in for example cardiovascular diseases.

Disclosure of Interest: None declared.

PO331-TUE

Microparticle level and origin during progression of normal pregnancy: a longitudinal study in healthy women

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Background: Microparticles (MP) are actively involved in increased hypercoagulable reported both in normal pregnancies and in pregnancy diagnosed with placenta-mediated complications.

Aims: To study the origin and the levels of plasma MP and MP activity in a group of healthy women followed longitudinally during normal pregnancy.

Methods: Seventy-five healthy normotensive pregnant women were enrolled and blood samples were prospectively collected at three differ-

ent time points corresponding to: 1st trimester (T1), 2nd trimester (T2), 3rd trimester (T3) of pregnancy. A group of age (± 3 years) matched healthy non-pregnant women acted as controls. Both standard clotting parameters and MP of different origin were measured. MP were identified by size and annexin V- FITC labelling using flow-cytometer. Platelet-derived MP (P-Selectin+) were identified using CD62P-PE; Endothelial-derived MP (EMP) using CD62E-PC5; Leukocyte-derived (LMP) by CD45-PC5 and Tissue factor-bearing MP (TF+) by CD142-PE. Procoagulant activity of MP was assessed using the STA[®]Procoag PPL assay. Procoagulant MP activity was measured using the STA[®] Procoag PPL assay.

Results: The levels of total, platelet-, endothelial-, leukocyte-derived and tissue factor-bearing MP in normotensive non-complicated pregnancy are higher in T1 as compared to non-pregnant age-matched women. PPL clotting time was significantly shorter ($P < 0.01$), indicating increased procoagulant activity, in the three trimesters of pregnancy as compared to controls. The shortest median clotting time was found in the 3rd trimester [39 (31–49) s]. Regardless of the origin, MP levels gradually increase during pregnancy, with the highest values reached in T3.

Conclusion: MP levels gradually increase during pregnancy. All types of MP including TF+ present with the highest levels in T3 of normal pregnancy. These may contribute to the increased hypercoagulable observed, particularly in the last months of normal pregnancy.

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PO332-TUE

Circulating microparticles in cord blood of normal pregnancy and of pregnancy with preeclampsia (PET)

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Background: In women with pre-eclampsia (PET) extensive activation of endothelial cells, leukocytes and coagulation has been reported. In PET increased placenta microthrombi is a prevalent recurrent histological finding. It is proposed that a study of coagulation alterations in cord blood may be more informative than that found in maternal blood.

Aims: To evaluate microparticle (MP) subtypes in the maternal peripheral blood and in the venous cord blood of pregnant women with PET in labour compared to those of a group of healthy parturient women.

Methods: Thirty-two pregnant women in labour, 16 with PE and 16 healthy parturient, in which blood samples were obtained, immediately after delivery, from cord blood and from maternal peripheral blood were enrolled. Total MP were identified by size and annexin V labelling using flow-cytometer (AMP). Platelet-derived MP (PMP and P-Selectin+) were identified adding anti-CD61 and anti-CD62P monoclonal antibody, respectively; endothelial-derived MP (EMP) using anti-CD62E; leukocyte-derived (LMP) by CD45 and Tissue factor-bearing MP (TF+) by CD142. Procoagulant activity of MP was assessed using the STA[®]Procoag PPL assay.

Results: Total MP, P-Selectin+, LMP and TF+MP are increased in pregnancies complicated by PE compared with normotensive parturient women ($P < 0.05$ in all comparisons). PMP levels are decreased in PE than in healthy women ($P < 0.05$). No difference was seen in EMP levels between the two groups. The PPL clotting time was significantly shorter ($P < 0.05$) in PE compared with controls. When we analysed only venous cord blood, all MP detected were significantly higher in

PE than in healthy normotensive parturient women ($P < 0.05$ in all comparisons).

Conclusion: We confirmed the role of MP in the hypercoagulable and pro-inflammatory intravascular reaction during PET. Larger prospective studies are needed to better define the clinical utility of MP measurement in peripheral and cord blood in PET.

Disclosure of Interest: E. Campello: None declared, L. Spiezia: None declared, C. Radu: None declared, S. Dhima: None declared, S. Visetini: None declared, S. Gavasso: None declared, B. Woodhams Shareholder of: Haemacon Ltd, E. Cosmi: None declared, P. Simioni: None declared.

Natural anticoagulants

PO333-TUE

A gain-of-function variant of protein C manifests itself as type II protein C deficiency in a healthy subject

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Background: In a recent cohort study we identified a healthy PC deficient subject whose PC antigen level of 65% and activity level of 50% (chromogenic assay) and 36% (clotting assay) were markedly low as observed in type II PC deficiency. The proband has a negative family history of venous thromboembolism (VTE). Genetic analysis revealed the proband has a heterozygous missense mutation in which Thr-315 of PC has been substituted with Ala (PC-T315A).

Aims: The aim of this study was to determine the molecular basis for this apparent type II PC deficiency in the subject.

Methods: PC-T315A was expressed in HEK-293 cells and its anticoagulant and antiinflammatory activities were compared to those of wild-type PC (PC-WT) in both activated and zymogenic forms. Established coagulation and cell-based assays were used to characterize properties of activated PC-WT (APC-WT) and APC-T315A. Same assays were used to analyze the activity of zymogens by coupling their activation by the thrombin-thrombomodulin (TM) complex to their enzymatic functions. for analysis of signaling activities of zymogens, a thrombin mutant (W215A/E217A) was used which has no activity toward PAR1, but can activate PC normally on endothelial cell surface.

Results: Similar to subject's plasma, a decrease in the activity of APC-T315A was observed in both purified and plasma-based assays. However, when anticoagulant assays were coupled to PC activation by thrombin-TM, PC-T315A exhibited improved anticoagulant activity. Similarly, coupling PC activation by thrombin-W215A/E217A on endothelial cells, resulted in higher cytoprotective activity for PC-T315A. Further analysis revealed Thr-315 is consensus N-linked glycosylation site for Asn-313 and that its elimination improves Vmax (~4–5-fold) of PC activation by thrombin-TM.

Conclusion: The improved activation of the PC zymogen by thrombin-TM may explain the basis for the proband's negative VTE pedigree. This variant is a gain-of-function mutant of PC that cannot be properly diagnosed by routine commercial kits.

Disclosure of Interest: None declared.

PO334-TUE

Evaluation of protein C levels in sickle cell disease subjects seen at the University of Benin Teaching Hospital, Benin City, NigeriaIheanacho O¹ and Nwogoh B²¹*Haematology and Blood Transfusion, University of Benin Teaching Hospital, Benin City, Edo State;* ²*Haematology and Blood Transfusion, University of Benin, Benin City, Edo State, Benin, Nigeria***Background:** Sickle cell disease (SCD) is a hypercoagulable state. The role of natural inhibitors of coagulation in the pathogenesis of SCD has not been fully investigated in our environment.**Aims:** This study was aimed at evaluating the levels of Protein C (PC) antigen and activity in sickle cell disease subjects; and to determine if any, the association between PC levels and parameters such as sex, age, ABO and Rhesus D blood groups in the study subjects.**Methods:** This is a cross sectional study conducted at the University of Benin Teaching Hospital, Benin City. A total of 29 consenting SCD subjects were recruited through the Paediatric and Adult haematology Clinics by simple random selection. Protein C antigen and activity levels were determined using immunological and functional assays respectively. Blood groups were determined using standard antisera whereas the Hb phenotypes of the participants were already predetermined.**Results:** The mean age of the subjects was 15.2 ± 2.4 years, 19 (65.5%) were <18 years and 15 (51.7%) were males. Eighteen (62.1%) were blood group O and 26 (89.7%) were Rh D positive. The average levels of PC Antigen and Activity were (65.7 ± 4.9) and (48.8 ± 2.8) respectively. Protein C antigen was significantly higher in subjects above 18 years of age ($P = 0.001$). There was no significant association between PC levels and parameters such as sex, age, ABO and Rh D blood group system.**Conclusion:** Protein C levels were reduced in the SCD subjects and functional PC (activity) deficiency was more prevalent in them. There was no significant association between PC levels and the assessed parameters (sex, age, ABO and Rh D blood group system).**Disclosure of Interest:** None declared.

PO335-TUE

Possible mechanism of activated protein C-induced inhibition of osteoclast differentiationAkita N¹, Yoshida K², Okamoto T³, Asanuma K², Nishioka J⁴, Suzuki K⁵ and Hayashi T⁶¹*Faculty of Medical Engineering, Suzuka University of Medical Science, Suzuka-city;* ²*Department of Orthopaedic, Mie University Graduate School of Medicine;* ³*Molecular Pathobiology and Cell Adhesion Biology, Mie University, Tsu-city;* ⁴*Department of Clinical Nutrition;* ⁵*Faculty of Pharmaceutical Science, Suzuka University of Medical Science, Suzuka-city;* ⁶*Department of Biochemistry, Mie Prefectural College of Nursing, Tsu-city, Japan***Background:** Activated protein C (APC) is an important serine protease which inactivates the blood coagulation cofactors, factor Va and VIIIa. So far, we have demonstrated that APC promotes the osteoblast proliferation via EPCR, and recently, that APC inhibits the differentiation of osteoclast precursor cells into osteoclast via EPCR.**Aims:** The purpose of this study is to elucidate the detailed molecular mechanism of inhibitory action of APC on the differentiation of osteoclast precursor cells into osteoclast.**Methods:** The effects of APC, protein C (PC) and S1P receptor agonist (FTY720) on the osteoclast differentiation were examined as follows: Normal human osteoclast precursor cells were cultured in their growth medium including soluble RANKL, M-CSF, and FBS, and on day 4and 7, the culture medium was replaced with same medium containing various concentration of APC, PC or FTY720 without FBS. On day 8, TRAP-positive multinucleated cells (≥ 3 nuclei) were counted manually using a light microscope. The effects of PC, anti-EPCR antibody, anti-PAR-1 antibody, anti-apoER2 antibody and recombinant apoER2 on the APC-induced inhibition of osteoclast differentiation were examined by mixing them with APC, and treating on day 4 and 7, and then counting the number of osteoclast in a similar manner on day 8.**Results:** APC dose-dependently inhibited the osteoclast differentiation, but PC and FTY720 did not exhibit inhibitory activity. PC dose-dependently interfered with the APC-induced inhibition of osteoclast differentiation. Similar to anti-EPCR antibody anti-PAR-1 antibody, anti-apoER2 antibody and recombinant apoER2 blocked the APC-induced inhibition of osteoclast differentiation.**Conclusion:** Our results suggested that the APC-induced inhibition of osteoclast differentiation is also mediated by PAR-1 and apoER2, along with EPCR on cell membranes, and these receptors are correlated with APC-induced inhibition of the osteoclast differentiation in a collaborative manner.**Disclosure of Interest:** None declared.

PO336-TUE

Pharmacokinetic study of the novel anticoagulant peptide variegins using LC-MS/MS, chromogenic substrate and modified thrombin time assaysShih N^{1,2} and Kini RM^{1,2}¹*Department of Biological Sciences;* ²*NUS Graduate School for Integrative Sciences & Engineering (NGS), National University of Singapore, Singapore, Singapore***Background:** Anticoagulants are used for treatment of thromboembolic disorders. Although effective, anticoagulants such as heparin or warfarin have many limitations, for example a narrow therapeutic window and risk of excessive bleeding. This calls for the development of effective anticoagulants without these limitations. Previously, we isolated the variegins peptide from the saliva of the tropical bont tick (*Amblyomma variegatum*). The variegins peptide was shown to be a high affinity direct thrombin inhibitor (10 pM) and an effective anticoagulant in a mouse thrombosis model, with no risk of excessive bleeding. We have developed an LC-MS/MS, chromogenic substrate and modified thrombin time assays for variegins quantitation in PK studies.**Aims:** Development of an LC-MS/MS, chromogenic substrate and modified thrombin time assays for variegins PK studies.**Methods:** PK studies. Variegins was administered to pigs and blood samples collected over-time.

LC-MS/MS assay. Plasma samples were extracted by solid phase extraction and quantified by single reaction monitoring.

Modified thrombin time assay. Plasma samples were added to a thrombin time assay and monitored at absorbance 650 nm.

Chromogenic substrate assay. Plasma samples were acid-treated and added to an assay with thrombin and S2238 substrate, and monitored at absorbance 405 nm.

Results: The LC-MS/MS assay has a linear range from 78.1 to 20,000 ng mL⁻¹ variegins plasma concentration. The modified thrombin time and chromogenic substrate assays, each comprising several sub-assays with different thrombin concentrations, cover an overall quantitation range of 19.5 to 20,000 and 39.1 to 20,000 ng mL⁻¹ of variegins plasma concentration, respectively. Using a non-compartmental analysis, the elimination half-lives of variegins obtained from the PK study using the LC-MS/MS, modified thrombin time and chromogenic substrate assays were 52.3 ± 4.4 , 50.4 ± 5.9 and 70.4 ± 6.6 min, respectively.**Conclusion:** The LC-MS/MS and modified thrombin time assays can be used for variegins PK studies.**Disclosure of Interest:** None declared.

PO337-TUE

Thrombomodulin inhibits tissue factor mediated thrombin generation in blood and prothrombin complex concentratesFareed J¹, Hoppensteadt D¹, Syed D¹, Kahn D¹, Mosier M² and Kaul I³¹Pathology; ²Medicine, Loyola University Medical Center, Maywood; ³Asahi Kasei, Waltham, USA

Background: A recombinant thrombomodulin (Recomodulin, Asahi Kasei, Tokyo, Japan) is currently used for the management of DIC and related disorders. Beside inhibiting thrombin and forming complexes with this enzyme, Recomodulin (RM) mediates its therapeutic effects involving modulation of inflammatory processes and thrombogenesis.

Aims: The purpose of this study is to determine the effect of RM on thrombin generation in plasma and prothrombin complex concentrates.

Methods: RM 12,800 (2.02 mg) ampules were commercially obtained. Working solutions of buffered RM were prepared at 100 µg mL⁻¹. Tissue factor mediated generation of thrombin and its inhibition in normal human plasma (NHP) (*n* = 25), platelet rich plasma (PRP) (*n* = 25) and various prothrombin complex concentrates such as Beriplex, Cofact, Feiba, Konyne, Octaplex, Preconativ, Profilin, Prothromplex (1 µg mL⁻¹) were investigated utilizing a fluorometric thrombokinetics method. In addition, thrombin generation markers such as F1.2 and TAT complex were also measured. The effect of RM on thrombin mediated fibrinokinetics was also measured using an optical method.

Results: RM produced a concentration dependent inhibition of thrombin generation in all systems. In the NHP the IC₅₀ was 0.29 ± 0.06 µg mL⁻¹ in contrast to PRP where the IC₅₀ was 1.99 ± 0.31 µg mL⁻¹. The inhibition of thrombin generation in various PCCs was also concentration and product dependent. Thrombin generation markers were also inhibited. Marked inhibition was noted in Preconativ, Prothromplex and Beriplex. RM also inhibited the fibrin formation in both the PRP and NHP system in a differential manner.

Conclusion: These results suggest that beside the direct inhibition of thrombin, RM is capable of inhibiting thrombin generation in a matrix dependent fashion. Moreover, RM is also capable of modulating fibrin formation as evident by fibrinokinetic studies.

Disclosure of Interest: J. Fareed: None declared, D. Hoppensteadt: None declared, D. Syed: None declared, D. Kahn: None declared, M. Mosier: None declared, I. Kaul Employee of: Asahi Kasei.

PO338-TUE

Heparanase activates antithrombin through the binding to its heparin binding site

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Background: Heparanase (HPase) is an endoglycosidase that participates in morphogenesis, tissue repair, heparan sulfates turnover and immune response processes. It is over-expressed in tumor cells favoring the metastasis as it penetrates the endothelial layer that lines blood vessels and facilitates the metastasis by degradation of heparin sulfates proteoglycans (HSPG) of the extracellular matrix. Furthermore, HPase cleaves the sequence in HSPG that is recognized by antithrombin (AT) leading to its activation.

Aims: To explore the interaction of HPase with AT and the potential pathological consequences of such interaction.

Methods: Purified proteins were used to evaluate the interaction between AT and HPase by SDS-PAGE and native gels. Anti-FXa and anti-FIIa activities of AT in presence of HPase were analyzed using chromogenic substrates and SDS-PAGE. R47C and P41L AT mutants were used to determine the binding site for HPase. Intrinsic fluorescence measurement was performed to evaluate the conformational change in AT provoked by the binding of HPase and the *K_d*.

Results: HPase interacts with AT in a non-covalent way. This interaction provokes the activation of AT but mainly increasing the anti-Xa inhibition, supporting an allosteric activation effect. HPase binds to the heparin binding site of AT as the activation of P41L and R47C mutants is impaired as compared to wild type AT. Intrinsic fluorescence analysis showed that HPase induces an activating conformational change in AT similar to that induced by heparin and with a *K_d* of 1.32 nM.

Conclusion: We demonstrate that HPase interacts with AT and activates its inhibitory function. However, this effect could only happen in physiological conditions in which HPase does not exert endoglycosidase activity and the expression is low. Only under hypoxia and low pH conditions, HPase is able to degrade heparan sulfate proteoglycans. The physiological and pathological relevance of this interaction is still under investigation.

Disclosure of Interest: None declared.

PO339-TUE

Antithrombin binds to and enhances the barrier function of huvecs independently of cell-surface heparan sulfate proteoglycansSaller F¹, Pautus S¹, Bourti Y¹, De Carvalho A¹, Abache T², Bianchini E¹, Plantier J-L² and Borgel D¹¹INSERM U1176 & UMR 1176 Univ. Paris Sud, Le Kremlin-Bicêtre; ²Direction de l'Innovation Thérapeutique, LFB Biotechnologies, Loos, France

Background: Antithrombin (AT) has been shown to be protective in a model of endothelial barrier disruption by LPS in EA.hy926 cells, through unknown mechanisms. However, this effect appears to be inhibited by fondaparinux (Fpx) and mediated by binding to heparan sulfate (HS) proteoglycans.

Aims: To further explore the barrier-enhancing effects of recombinant human AT (rhAT) in HUVECs and their dependency on HS proteoglycans.

Methods: rhAT was expressed in Freestyle™ 293-F cells and the endothelial barrier function of HUVECs was investigated using a real-time impedance-based cell monitoring system (iCELLigence, ACEA Biosciences) on non-treated or TNF-treated monolayers. The binding of rhAT to HUVECs was measured by ELISA on cell monolayers fixed with 4% paraformaldehyde.

Results: Addition of 750 µg mL⁻¹ rhAT to HUVEC monolayers induced: 1) a rapid (within minutes) increase in the Normalized Cell Index (NCI) values, that peaked at ~45 min and returned back to control levels at 4 h; 2) a late increase in the NCI starting at 12 h and persisting at 48 h. Interestingly, pre-incubation of rhAT with saturating concentrations of Fpx (25 µM) had no effect on these rhAT-dependent increases in the NCI. The stimulation of HUVEC monolayers with 1 ng mL⁻¹ TNF induced a drop in the NCI that peaked at ~20 h post-TNF and was followed by a late recovery phase. The drop in the NCI was diminished and the recovery phase was accelerated when HUVECs were pre-treated with rhAT for 4 h, either in the absence or presence of Fpx. Furthermore, rhAT (0–750 µg mL⁻¹) saturably bound to HUVECs in our ELISA (*K_{d app}* = 337.7 ± 21.7 nM) and the binding of 750 µg mL⁻¹ rhAT was not inhibited by Fpx (2.5–250 µM). In addition, the binding of rhAT was not impaired (*K_{d app}* = 127.2 ± 9.8 nM) when HUVECs were cultured with 50 mM NaClO₃.

before fixation in order to reduce sulfation of the HS chains on HU-VECs.

Conclusion: In the present study, AT was shown to bind to HUVECs and to exert some barrier-enhancing effects with no apparent requirement for AT binding to HS.

Disclosure of Interest: None declared.

PO340-TUE

Tetramethylpyrazine decreases Receptor for Advanced Glycation End-products (RAGE) via suppression of Foxa2 and induction of nos in HepG2 cells

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Background: Diabetic liver damage results from hyperglycemia followed by increasing formation of advanced glycosylation end products (AGEs), which leads to cardiovascular morbidity is dependent of receptor for AGEs (RAGE). Tetramethylpyrazine, an active constituent of Chinese herb, possesses anti-coagulant, anti-inflammatory, anti-angiogenic, and anti-oxidant activities. However, the effect and possible mechanisms of tetramethylpyrazine on RAGE responses remain unclear.

Aims: To explore whether tetramethylpyrazine decreases AGEs-induced RAGE expression, and if this occurs through Foxa2 downregulation and NOS activation.

Methods: Transcriptional and protein levels were analysed by real-time PCR and Western blots. The Foxa2 was over-expressed by plasmid, NOS was silenced by short hairpin RNA and NOS activity inhibitor NG-methyl-L-arginine (LNMA), respectively.

Results: Tetramethylpyrazine significantly reduced the expression of RAGE at both mRNA and protein level in HepG2 cells. Decreased forkhead box protein A2 (foxa2) and increased NOS were confirmed in the meantime. Additionally, overexpression of Foxa2, NOS shRNA and LNMA markedly abated the inhibitory effects induced by tetramethylpyrazine on RAGE expression in HepG2 cells.

Conclusion: Tetramethylpyrazine decreases RAGE via suppression of Foxa2 and induction of NOS in HepG2 cells, suggesting a novel potential mechanism for tetramethylpyrazine in hyperglycemia.

Disclosure of Interest: None declared.

PO341-TUE

Quantitative transcriptome and proteome analysis of dermacentor reticulatus salivary glands for the identification of novel anticoagulants and immunomodulatory proteins

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Background: The ability of hematophagous arthropods to feed successfully on blood has evolved several times in various arthropod clades.

At each stage of evolution, novel solutions to counter the problem posed by host hemostasis and immune system were generated. Hence, the saliva of blood-feeding arthropods is an excellent source of anti-hemostatic and immunomodulatory molecules. *Dermacentor reticulatus* (ornate cow tick) is a hard tick found in Europe, that feeds on the blood of cattle, dogs and human. It has been hypothesized that for successful feeding, ticks produce antithrombotic and immunomodulatory proteins during the course of feeding.

Aims: To identify novel anticoagulants and immunomodulatory proteins from the salivary gland extracts of *D. reticulatus* at different stages of feeding and develop them as antithrombotic drugs and anti-tick vaccines.

Methods: The transcriptome of male and female tick SGEs was sequenced by Illumina sequencing. The proteome of male and female ticks SGEs were quantitatively analyzed with iTRAQ.

Results: We have identified about 50,000 transcripts in the transcriptome and more than 700 proteins in the proteome of the male and female ticks at different stages of feeding. We have quantified these proteins and further grouped them into functional classes with a special focus on anticoagulants and immunomodulatory proteins. Among the antithrombotic proteins, the major classes that we have identified are kunitz and kazal inhibitors, lipocalins, serpins, cystatins, hirudin and variegin like proteins. The immunomodulatory proteins we have identified are immunoglobulin binding proteins, evasins, cytokine and chemokine inhibitors. We are further exploring some of these proteins by recombinant expression to study their structure-activity relationships.

Conclusion: With limited amounts of tick salivary gland extracts, we have successfully generated a library of proteins that can serve as a platform for development of novel antithrombotic and anti-tick vaccines.

Disclosure of Interest: None declared.

PO342-TUE

Similar responses of phosphatidylserine and phosphatidylethanolamine towards cofactor activity of human protein Z, a natural anticoagulant

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Background: Protein Z (PZ) is a natural anticoagulant that acts as a cofactor of a serpin, protein Z dependent protease inhibitor (ZPI), which is a fXa inhibitor. PZ binds ZPI with high affinity and accelerates the rate of inhibition by more than 1000-fold in the presence of Ca²⁺ and phospholipids. In humans, PZ deficiency is suggested to be associated with ischaemic risk and thrombotic complications.

It is evident from various studies that PZ promotion of the fXa inhibition results from the anchoring of its Gla domain onto phospholipid surfaces and positioning the bound ZPI into close proximity of the Gla-anchored fXa with the formation of a ternary complex of PZ-ZPI-fXa. Interaction of PZ with phospholipid membrane thus appears to be absolutely crucial for its cofactor activity. We hypothesize that molecular phosphatidylserine (PS) and possibly molecular phosphatidylethanolamine (PE), exposed during platelet activation, regulate cofactor activity of PZ.

Aims: In order to prove the hypothesis we aimed to study the interaction of fXa-PZ-ZPI in presence of molecular PS and PE.

Methods: We have used six carbon chains soluble phospholipids (C6PL) as a tool to study the effect of molecular phospholipids employing various biophysical techniques and chromogenic assays.

Results: Preliminary studies revealed that both PS and PE bind to PZ with equal affinities (kd ~ 49 μM) and binding was observed to be synergistic. Anisotropy experiments showed similar effects of PS and PE towards the interaction of PZ with fXa. Comparable effects of PS and PE on PZ mediated antithrombin inhibition of fXa were also

observed. We noticed an increase in rate of PZ/ZPI mediated inhibition of fXa in presence of either PS or PE, however, the efficacy is not as high as that in presence of membrane. Weak interactions of PZ with few other lipids were observed but that did not contribute to the cofactor activity of PZ.

Conclusion: The interaction of PZ with phospholipids is independent of any specificity towards PS or PE.

Disclosure of Interest: None declared.

PO343-TUE

The effect of age on glycan microheterogeneity of human Alpha-2-macroglobulin

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Background: Alpha-2-macroglobulin (a2 m) is a plasma glycoprotein serine protease inhibitor. Previous studies have shown a2 m levels to be twice as high in newborns vs. adults. This may contribute to resistance towards thrombotic events in children. Protein glycosylation is known to affect pharmacodynamics and function. We previously found that newborn and adult a2 m have no differences in N-linked glycan microheterogeneity. However, differences in a2 m microheterogeneity between age groups have yet to be elucidated.

Aims: To examine differences in glycan microheterogeneity between newborn and adult a2 m.

Methods: To evaluate sialic acid (SA) content, newborn and adult plasma samples were incubated with Neuraminidase (*Clostridium perfringens*) and subjected to native polyacrylamide gel electrophoresis (PAGE) and western blotting (WB). To detect any non-sialylated terminal galactose residues, plasma samples were incubated with immobilized *Ricinus communis* (RCA₁₂₀). Lectin-bound and unbound fractions were separated and subjected to SDS-PAGE and WB. To evaluate the affinity of a2 m for ricin, bound proteins were eluted with varying concentrations of galactose and released a2 m was detected by SDS-PAGE and WB.

Results: Following treatment with Neuraminidase, newborn a2 m exhibited slightly greater change in migration compared to adult, though the difference was not statistically significant. Additionally, newborn a2 m exhibited a higher percentage of molecules bound to RCA₁₂₀ than adult, however this was not statistically significant. Elution of a2 m from RCA₁₂₀ with galactose produced similar profiles for newborn and adult molecules.

Conclusion: This is the first study investigating glycan microheterogeneity between newborn and adult a2 m. The results suggest no major difference in microheterogeneity in terminal SA or galactose content between the two molecules. Similarity in a2 m glycosylation between age groups may suggest structural features that are critical throughout life.

Disclosure of Interest: None declared.

PO344-TUE

Quantitative immunoassay for the measurement of activated protein C complexes with Alfa-2-macroglobulin (APC:α₂M)

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Background: We previously identified *in vitro* plasma complexes between activated protein C and α₂-macroglobulin (APC:α₂M) by immunoblotting.

Aims: To set up an ELISA for the measurement of APC:α₂M in plasma samples.

Methods: Blood was anticoagulated with hirudin (300 U mL⁻¹). Hirudinised plasma was incubated with 2 and 10 μg mL⁻¹ APC (final concentration). At time intervals, aliquots were withdrawn and complexes of APC with protein C inhibitor (PCI), α₁-antitrypsin (α₁AT) and α₂M were quantified with homemade ELISAs. for the APC:α₂M assay, samples were pre-treated with dithiothreitol and iodoacetamide to expose the epitopes of the APC bound to α₂M. A monoclonal anti-PC antibody (Ab) was used as capture Ab and a polyclonal anti-α₂M Ab bound to peroxidase as detection Ab.

Results: The range of the APC:α₂M assay was 1–210 ng mL⁻¹ of complexed APC, with intra- and inter-assay CVs of 6–18% for the whole range. By adding 2 μg mL⁻¹ APC to plasma, PCI and α₁AT were the major inhibitors of APC (0.68 and 0.55 μg mL⁻¹ of complexed APC, respectively, after a 3 h incubation), whereas at 10 μg mL⁻¹ of APC, α₁AT and α₂M were the major APC inhibitors (3.1 and 2.6 μg mL⁻¹, respectively), probably because of PCI depletion by an excess of APC. Citrate plasma from patients with severe sepsis showed up to 10 ng mL⁻¹ of APC complexed to α₂M, whereas the normal pool plasma had about 0.5–1 ng mL⁻¹.

Conclusion: α₂M may become a major APC inhibitor during infusion of high APC concentrations as may occur in the near future in the treatment of patients with several inflammatory diseases. This APC: α₂M assay may be useful to measure complex levels in plasma from patients and controls and to ascertain the fate of infused APC during APC treatment. ISCHII (PI12/00027, RD12/0042/0029, PI14/00512, PI14/00079, FI14/00269), FEDER, Generalitat Valenciana (Prometeo 2015). SN is an IIS La Fe researcher. PM is a Miguel Servet researcher (FIS-CP09/00065).

Disclosure of Interest: None declared.

PO345-TUE

Identification of a novel mutation p.K234Efs*24 in the PROC gene and a phenotype-genotype characteristics of thai patients diagnosed with hereditary protein C deficiency and symptomatic thromboembolism

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Background: Protein C (PC) deficiency is a major inheritable risk factor for venous thromboembolism (VTE) in the Asian populations. Recent genetic analyses of PC gene revealed a significant increase in risk of VTE in patients carrying mutant alleles. However, the phenotype-genotype characteristics of PC deficiency in Thai population remain unknown.

Aims: To study the genetic and clinical characteristics of Thai patients diagnosed with protein C deficiency.

Methods: A retrospective chart review was performed in eight symptomatic, unrelated patients diagnosed with PC deficiency who also had the PROC gene mutation analysis.

Results: The median age was 28 years (range 23–47). Half of the patients were females. VTE was the most common manifestation (87.5%). Only one patient had ischemic stroke. Unusual sites of VTE, including cerebral venous sinus thrombosis and splanchnic vein thrombosis were found in 5 patients (71.4%). The mean PC activity was 37.7 ± 11.6 IU dL⁻¹. Four different mutations in the PROC gene, including one novel variant were identified. The most frequent mutation was PROC p.Arg189Trp (62.5%). Other mutations were p.Ala178Pro (25%), p.Asn384Lys (12.5%), and a novel variant p.K234Efs*24 (12.5%). Four patients (50%) were homozygotes, including one patient with compound heterozygote of p.Arg189Trp and p.Asn384Lys. The mean PC activities in the homozygotes and heterozygotes were 32.1 ± 10.0 IU dL⁻¹ and 46.9 ± 8.0 IU dL⁻¹,

respectively. The median duration of oral anticoagulant therapy was 8 months (range 1–43). Among five patients (62.5%) who discontinued anticoagulant therapy, there was only one patient with recurrent VTE.

Conclusion: The novel mutation p.K234Efs*24 was identified in the *PROC* gene. The *PROC* p.Arg189Trp mutation was the most frequent mutant allele. Further study is needed to clarify the relationship between the genetic background and the predisposition of thromboembolic disease.

Disclosure of Interest: None declared.

PO346-TUE

The PDI inhibitor isoquercetin reduces thrombin generation in healthy adults and in patients with elevated antiphospholipid antibodies

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Background: Protein disulfide isomerase (PDI) regulates thrombus formation following vascular injury in animal models. Previous studies identified 3-O-glycosylated quercetin flavonoids as potent inhibitors of PDI activity *in vitro* and *in vivo*. However, the appropriate dosing of isoquercetin for clinical use and the effect of PDI inhibition on thrombin generation in humans are unknown.

Aims: To determine whether oral isoquercetin inhibits PDI activity and thrombin generation in healthy adults and in patients with elevated antiphospholipid antibodies.

Methods: We performed a dose-escalation study of isoquercetin at 500 mg ($N = 10$) and 1000 mg ($N = 16$) in healthy volunteers and dose expansion (1000 mg) in patients with persistently elevated antiphospholipid antibodies ($N = 5$). PDI activity was measured in plasma using an eosin probe coupled to glutathione. Thrombin generation was measured using a thrombin-induced thrombin generation assay.

Results: Isoquercetin (500 mg) demonstrated maximum PDI inhibition ($59 \pm 19\%$, $P = 0.001$) at 4 h post-ingestion and normalized by 6 h. Following ingestion of 1000 mg isoquercetin, PDI inhibitory activity in plasma reached maximum inhibition at 8 h post-ingestion ($57 \pm 14\%$, $P = 0.004$), and remained decreased at 24 h post-ingestion ($36 \pm 11\%$, $P = 0.04$). To assess functional consequences of PDI inhibition by isoquercetin, platelet-dependent thrombin-generation was evaluated in a second cohort of patients. Ingestion of isoquercetin (1000 mg) resulted in a $68 \pm 14\%$ ($P < 0.05$) decrease in platelet-dependent thrombin generation and a $61 \pm 21\%$ ($P = 0.002$) reduction in PDI inhibition. All 5 patients with elevated antiphospholipid antibodies demonstrated inhibition in PDI activity ($90 \pm 9\%$) and thrombin generation ($66 \pm 8.2\%$) at 4 h ($P < 0.05$).

Conclusion: The oral PDI inhibitor, isoquercetin, demonstrates significant inhibition of PDI activity and thrombin generation in both healthy adults and patients with elevated antiphospholipid antibodies.

Disclosure of Interest: None declared.

PO347-TUE

Investigation protein S deficiency induced purpura fulminans

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Background: Complete Protein S (PS) deficiency induces lethal skin necrosis and disseminated intravascular coagulation known as Purpura Fulminans (PF). The pathophysiology of PF is uncertain although the imbalance between pro/anticoagulant factors is thought to be the etiological factor. Recently, our knowledge on PS increased, indicating that beyond its role in coagulation, PS has also cellular functions.

Aims: Elucidate PF pathophysiology using different PF mouse models.

Methods: An inducible *ProS* gene silencing system (polyI:C- inducible Mx1-Cre) was generated (*ProSlox/loxMx1Cre+*, *ProSlox/-Mx1Cre+*) to decrease PS levels. 0.8 mg day⁻¹ of warfarin was administered to *ProS*^{+/−} mice to reduce PS. To evaluate the vasculature defects, immunostainings of whole mounted dorsal skin from *ProS*^{−/−} embryos (E15) were performed.

Results: Ten days after polyI:C treatment, PS levels was significantly reduced: $47.7 \pm 6\%$ in *ProSlox/loxMx1Cre+* and $14.5 \pm 7\%$ in *ProSlox/-Mx1Cre+*. However, no skin lesions compatible with PF were observed during 2 months observation. With warfarin treatment, early lesions were erythematous with highly visible ear skin vessels, vascular engorgement, intradermal edema and rare intravascular-thrombosis. In advanced lesions we noticed massive RBC extravasation, intra-epidermal hemorrhagic blisters and necrotic areas. CD31 staining showed areas with underdeveloped and less dense vascular networks, collapsed vessels and less vessels branch points in *ProS*^{−/−} as compare to wt. Observed RBC extravasation indicate a vascular leakage (Ter119, CD144). Investigation of the lymphatic vessels revealed massively enlarged vessels (Lyve1) and increased macrophages number (F4/80) in *ProS*^{−/−}.

Conclusion: In *ProS*^{−/−} embryos, the vasculature defects point toward an involvement of the vascular wall during PF. Furthermore, the enlarged lymphatic vessels and increased number of macrophages let us suspect a prominent inflammatory process. Experiments are ongoing to better characterize the vasculature defects and inflammation in *ProS*^{−/−}.

Disclosure of Interest: None declared.

PO348-TUE

Quantitative platelet TFPI-alpha release is not influenced by sex and oral contraceptive use

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Background: Platelets contain 5–10% of circulating TFPI α (TFPI) and in contrast to plasma TFPI, the platelet TFPI pool exclusively consists of full length TFPI. As 80% of plasma TFPI is C-terminally truncated and does not play an anticoagulant role in blood coagulation, local release of platelet TFPI α at the site of injury is likely to play an important regulatory role in thrombin generation and localization of thrombus formation.

Aims: Determine the amount and anticoagulant activity of full length TFPI released from platelets in males, females, and oral contraceptive users.

Methods: Blood samples were obtained from 30 healthy volunteers (10 males, 10 females, and 10 females using oral contraceptives (OC)). Platelet TFPI was released in platelet rich plasma (PRP) and in platelet isolates using convulxin and thrombin, respectively, and quantified by free TFPI ELISA and thrombin generation assays.

Results: In healthy volunteers, mean plasma TFPI levels decreased significantly from men (0.31 ± 0.05 nM) to women (0.18 ± 0.05 nM) to women using OC (0.12 ± 0.02 nM). Remarkably, no differences were found in platelet-released TFPI in PRP among subgroups (0.06 ± 0.03 nM). Release of platelet TFPI in convulxin activated PRP strongly reduced thrombin generation in a subsequent preparation of PPP, compared to parent PPP. TFPI-release from washed platelets adjusted to an identical platelet count of $250 \times 10^9 \text{ L}^{-1}$ did not differ significantly between subgroups with a mean of 0.11 ± 0.02 nM.

Conclusion: Platelets release similar amounts of TFPI regardless of plasma TFPI concentrations and unaffected by sex or OC use. We speculate that platelet TFPI is important to prevent systemic coagulation and thrombosis and restrict thrombus formation to the site of the growing platelet plug. The stable contribution of platelet TFPI to the anticoagulant potential in plasma is likely to become particularly relevant under conditions of low plasma TFPI levels with OC use.

Disclosure of Interest: None declared.

PO349-TUE

Role of tissue factor pathway inhibitor-alpha in platelet-dependent fibrin formation under flow

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Background: Tissue factor pathway inhibitor-alpha (TFPI) is a Kunitz-type serine protease inhibitor that suppresses coagulation by inhibiting tissue factor/factor VIIa (TF/FVIIa) and FXa. In plasma from patients with haemophilia A, inhibition of TFPI has been shown to fully normalize FXa and thrombin generation and may provide a novel way of haemophilia treatment. However, the role of TFPI in fibrin clot formation under flow has remained under-investigated.

Aims: To determine the role of TFPI in regulating coagulation and fibrin formation under venous flow conditions.

Methods: Platelet aggregation and fibrin formation were quantified in real-time, using a parallel-plate flow device by perfusing whole-blood or plasma over microspots containing collagen and varying amounts of TF. Haemostatic effects were compared under venous flow conditions and under stasis. A fluorescence thrombin substrate was used to monitor in-situ thrombin generation.

Results: Following platelet aggregation, the onset of growing fibrin fibres was observed by confocal microscopy and defined as fibrin formation time (FFT). Inhibition of TFPI in normal blood or plasma significantly shortened the FFT, but only in the presence of limited amounts or in the absence of TF. Markedly, inhibition of TFPI caused a more prominent decrease in FFT in haemophilia A plasma compared to normal plasma, which persisted on surfaces containing high amounts of TF. Under conditions of flow arrest, thrombin generation on procoagulant platelets followed by fluorescence microscopy was increased upon TFPI inhibition, explaining the effect on fibrin formation.

Conclusion: Under venous flow, TFPI inhibits platelet-dependent fibrin formation at low concentrations of TF, which becomes more prominent in the absence of FVIIIa.

Disclosure of Interest: None declared.

Non-vitamin K antagonist oral anticoagulants II

PO350-TUE

Association rate constants rationalize the sensitivity of prothrombin time and thrombin generation assay to apixaban and rivaroxaban

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Background: Rivaroxaban and apixaban are non-vitamin K antagonist oral anticoagulants. They are both selective and direct factor Xa (FXa) inhibitors approved in the prevention and treatment of thromboembolism. Surprisingly, contrary to rivaroxaban, prothrombin time (PT) is little sensitive to clinically relevant concentration of apixaban.

Aims: We investigated the existing pharmacodynamic discrepancy between apixaban and rivaroxaban.

Methods: We compared kinetics of FXa inhibition in strictly identical conditions (pH 7.48, 37 °C, 0.15 M) to estimate the inhibition (K_i) and association rate (k_{on}) constants. We evaluated the impact of these kinetic constants through numerical integration techniques simulating PT and thrombin generation assay (TGA). Predicted results were compared to corresponding PT or TGA triggered in plasma spiked with apixaban or rivaroxaban.

Results: K_i values of 0.74 ± 0.03 and 0.47 ± 0.02 nM and k_{on} values of $7.3 \pm 1.6 \cdot 10^6$ and $2.9 \pm 0.6 \cdot 10^7 \text{ M}^{-1} \text{ s}^{-1}$ were obtained for apixaban and rivaroxaban, respectively, suggesting that rivaroxaban neutralizes FXa four-fold more rapidly than apixaban ($P < 0.0001$). Simulation using these values predicted that in TGA triggered with 20 pM tissue factor, 100 ng mL⁻¹ apixaban or rivaroxaban increased 1.8- or 3.0-fold the lag time and 1.4- or 2.0-fold the time to peak, whilst decreasing 1.2- or 3.1-fold the maximum thrombin and 1.7- or 3.5-fold the endogenous thrombin potential. These figures were consistent with those obtained through TGA performed in plasma. Simulated PT ratios were also in accord with PT in plasma: slightly above (1.2-fold) the normal threshold with apixaban but largely above with rivaroxaban. Analogous differences in TGA and PT were obtained irrespective of the drug amount added.

Conclusion: The k_{on} values for FXa of apixaban and rivaroxaban rationalize the unexpected lower sensitivity of PT and TGA to the former.

Disclosure of Interest: G. Jourdi: None declared, V. Siguret Consultant for: Bristol-Myers Squibb and Bayer Healthcare AG, A.-C. Martin: None declared, J.-L. Golmard: None declared, A. Godier Consultant for: Bristol-Myers Squibb and Bayer Healthcare AG, C.-M. Samama Consultant for: Bristol-Myers Squibb and Bayer Healthcare AG, P. Gaussem: None declared, I. Gouin-Thibault Consultant for: Bristol-Myers Squibb and Bayer Healthcare AG, B. Le Bonniec: None declared.

PO351-TUE

Interaction of andexanet alfa, a universal antidote to fXA inhibitors, with tissue factor pathway inhibitor enhances reversal of fXA inhibitor-induced anticoagulation

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Background: Andexanet alfa (AnXa) is a modified, recombinant human fXA molecule developed to reverse the anticoagulant activity of fXA inhibitors in patients during episodes of major bleeding or before

emergency surgery. AnXa retains high affinity for fXa inhibitors and has no pro- or anticoagulant activity on its own. In preclinical toxicology and multiple clinical studies in healthy subjects, AnXa was well-tolerated and showed immediate onset of action. No significant interaction with plasma coagulation proteins has been observed, except for TFPI.

Aims: The objective was to further characterize the AnXa interaction with TFPI and fXa inhibitors.

Methods: AnXa interaction with TFPI was studied with or without a fXa inhibitor in enzymatic assays, in cell-based binding assays and TF (5 pM)-initiated thrombin generation (TG) assays in human plasma.

Results: AnXa binds to TFPI with an affinity similar to fXa ($K_{iXa-TFPI} = 0.03$ nM, $K_{dAnXa-TFPI} = 0.07$ nM). Unlike fXa-TFPI, however, the AnXa-TFPI complex does not inhibit fVIIa/TF enzymatic cleavage of a fluorogenic substrate (Z-VVR-AMC) or fX activation – a finding likely due to AnXa's lack of the Gla-domain. AnXa binds to fXa inhibitors, including rivaroxaban (Riva) with high affinity ($K_d = 0.5$ – 1.5 nM). AnXa binds to TFPI on HUVECs as evidenced by its partial blockade by a TFPI-K2 antibody. Furthermore, AnXa binding was almost completely blocked by Riva, indicating that co-administration with a fXa inhibitor could dampen the AnXa-TFPI interaction. AnXa alone in TG has minimal effect on ETP or peak thrombin, while Riva alone reduces both parameters dose-dependently with increasing Riva anti-fXa activity. With AnXa+Riva, however, the AnXa-TFPI interaction leads to enhanced TG at similar Riva anti-fXa levels.

Conclusion: Competitive interaction of AnXa with TFPI and fXa inhibitors could enhance TG and reversal of anticoagulation in bleeding patients at clinically relevant AnXa doses/regimens for fXa inhibitors.

Disclosure of Interest: G. Lu Employee of: Portola Pharmaceuticals, Inc., J. Lin Employee of: Portola Pharmaceuticals, Inc., G. Coffey Employee of: Portola Pharmaceuticals, Inc., J. Curnutte Shareholder of: 3-V Biosciences, Consultant for: Sea Lane Biotechnologies, Employee of: Portola Pharmaceuticals, Inc., P. Conley Employee of: Portola Pharmaceuticals, Inc.

PO352-TUE

Direct oral anticoagulants: our experience

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Background: The direct oral anticoagulants (DOA) Dabigatran, Rivaroxaban and Apixaban have predictable pharmacokinetic and pharmacodynamic profiles and are alternatives to warfarin. Some of them are available in Italy for treatment of venous thromboembolism (VTE).

Aims: We describe our experience in treatment with direct oral anticoagulants after 18 months from their approval in Italy.

Methods: From June 2013 to December 2014, 165 patients (87 males, 78 females), aged 18–95 (mean 71) received DOAs. Forty of them were in therapy with Dabigatran, 21 males, 19 females, aged 58–95 (mean 79); 47 with Rivaroxaban, 27 males, 20 females, aged 18–92 (mean 64); 78 with Apixaban, 39 males, 39 females, aged 60–92 (mean 79). One hundred and thirty-six patients had AF (40 with Dabigatran, 18 with Rivaroxaban, 78 with Apixaban), 29 patients received Rivaroxaban for treatment of thromboembolism. Of 40 patients with Dabigatran, 13 of them received 150 mg bid, 27 patients 110 mg bid. Of 47 patients with Xarelto, 43 of them received 20 mg, 4 patients 15 mg (these patients all suffering from atrial fibrillation). Of 78 patients with Apixaban, 50 of them received 5 mg bid, 28 patients 2.5 mg bid. The observational period was 1–18 months (mean 9).

Results: We reports adverse effects in 15 Patients: 13 of these were in treatment with Dabigatran (all of them with epigastric pain, appetite loss, stomatitis, one had also chest pain, another also had superficial

vein thrombosis), 2 were treated with Rivaroxaban (meno-metrorrhagia, bruising). No events was observed with Apixaban. Nine patients were undergoing to surgery (4 with Dabigatran, 2 with Xarelto, 3 with Apixaban) without complications.

Conclusion: In our patients DOA prescription are used more frequently in naive patients. Adverse drug reactions were frequent in patients treated with Dabigatran, specially gastrointestinal effects, so to require the suspension of the drug. In patients treated with other anticoagulants adverse events were rare.

Disclosure of Interest: None declared.

PO353-TUE

Clinical presentation, impact and course of major bleeding events with apixaban and warfarin

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Background: In the AMPLIFY trial, apixaban, a direct acting oral anticoagulant (DOAC), proved to be non-inferior to low-molecular-weight heparin (LMWH)/warfarin for treatment of venous thromboembolism, and was associated with significantly less bleeding. Information is needed on the presentation and clinical course of bleeding during therapy with DOACs.

Aims: In this post-hoc analysis, we blindly classified the seriousness of clinical presentation and course of all episodes of major bleeding in the AMPLIFY trial.

Methods: Three clinicians classified all major bleeding episodes, first independently and then by discussion, using pre-defined criteria. The first classification assessed the clinical impact at time of presentation. The second assessed the measures and interventions applied and the clinical course. Both classifications comprised four categories; category one being the mildest and four being the most severe.

Results: There were a total of 63 major bleeds; 14 with apixaban and 49 with LMWH/warfarin. A severe clinical presentation (category three or four) was observed in 28.5% of the apixaban and in 44.9% of the LMWH/warfarin associated major bleeding events (odds ratio 0.49, 95% CI 0.14–1.78). No major bleeding event from either group fit the criteria for the most severe clinical course (category four). A category three clinical course was observed in 14.3% of the apixaban and in 12.2% of the LMWH/warfarin associated major bleeding events (odds ratio 1.19, 95% CI 0.21–6.69).

Conclusion: There was a trend for less severe clinical presentation of major bleeding events in apixaban treated patients, compared with LMWH/warfarin treated patients. The clinical course was similar for apixaban and warfarin associated major bleeding events.

Disclosure of Interest: S. Bleker: None declared, A. Cohen Consultant for: Bayer, Boehringer-Ingelheim, BMS, Daiichi, GSK, Johnson and Johnson, Mitsubishi Pharma, Pfizer, Portola, Sanofi, Schering Plough, Takeda, X01, Paid Instructor at: Bayer, Boehringer-Ingelheim, BMS, Daiichi, GSK, Johnson and Johnson, Mitsubishi Pharma, Pfizer, Portola, Sanofi, Schering Plough, H. Büller: None declared, G. Agnelli: None declared, A. Gallus Consultant for: BMS, Pfizer, G. Raskob Consultant for: Daiichi Sankyo, Bayer, BMS, Boehringer Ingelheim, Isis Pharmaceuticals, Janssen Pharmaceuticals, Pfizer, Speaker Bureau

of: Bayer, Daiichi Sankyo, BMS/Pfizer, Janssen, J. Weitz: None declared, M. Curto Employee of: Pfizer Inc., M. Sisson Shareholder of: Pfizer Inc., Employee of: Pfizer Inc., S. Middeldorp: None declared.

PO354-TUE

Dabigatran prevents thrombosis with enhancement of secondary fibrinolysis in a mouse venous thrombosis model

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Background: Recent clinical trials have revealed that dabigatran etexilate (DE) shows a significant clinical benefit in decreasing the incidence of intracranial hemorrhage (ICH) compared with warfarin, despite their equal efficacy at preventing thrombosis.

Aims: This study aimed to elucidate the mechanisms of ICH reduction under new oral anticoagulant therapy, while preserving anti-thrombotic efficacy.

Methods: C57BL/6J mice received oral DE administration by gastric gavage, or were treated intraperitoneally with argatroban, a similar direct thrombin inhibitor. Plasma drug concentrations were measured by anti-thrombin activity. ICH was induced by the direct injection of collagenase into the striatum at several time points after treatment. Thrombus formation was triggered by ligation of the inferior vena cava (IVC). Both drugs were administered just after (prophylaxis model) or 24 h after IVC ligation (treatment model).

Results: After the oral administration of DE, peak plasma concentrations were reached in 30–60 min, then reduced rapidly. ICH volume induced 1 h after oral DE administration was largest, and its severity was observed to reduce as the duration from drug administration to ICH induction was extended. ICH severity after DE administration was identical to that after argatroban administration, as long as the anti-thrombin activity of the two treatments was the same. DE showed strong anti-thrombotic effects compared with argatroban, despite the fact that plasma anti-thrombin activities after DE administration were lower than those of continuous treatment during more than half of the observation period. Treatment with tranexamic acid completely extinguished the treatment efficacy of DE.

Conclusion: Our data suggest that fluctuations of drug concentrations ensure the safety of DE, and effectively prevent thrombosis through the activation of physiological fibrinolysis.

Disclosure of Interest: None declared.

PO355-TUE

Dabigatran and rivaroxaban adherence study- real world experience in different clinics and extended follow up (dariva study)

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Background: The degree of adherence with non-vitamin K antagonist oral anticoagulants (NOACs) in the absence of laboratory monitoring is unclear.

Aims: To compare adherence to rivaroxaban and dabigatran regimens among patients in different clinical settings.

Methods: In a prospective cohort study we included patients on rivaroxaban or dabigatran for at least 3 months followed at an anticoagu-

lation (AC) clinic, cardiology clinics and family medicine (FM) clinics in Canada and Sweden. We calculated the adherence from pharmacy refill data and in a smaller cohort of 75 patients also with a validated 8-item Morinsky Medication adherence scale. We present the preliminary adherence data from this cohort.

Results: of 474 patients (49% female; median age 73 years [range: 36–95]; 97% had atrial fibrillation, 3% had venous thromboembolism; 205 were on dabigatran and 269 on rivaroxaban. The mean duration on NOACs was 443 days. Median adherence was 100% (interquartile range [IQR] 97–100) for the entire cohort and for each clinic; IQR for cardiology clinics was 99–100, for FM clinics 90–100, and for the AC clinic 100–100. Adherence of $\geq 80\%$, typically considered as good, was seen in 95%, 89%, and 100% of patients followed at cardiology clinics, FM clinics and the AC clinic; it was 93% in Canada and 95% in Sweden. None of the differences are statistically significant. With the Morinsky Medication adherence scale all patients had medium adherence with an average score of 6.9 (standard deviation [SD] ± 1.29) with mean scores of 7.02 (SD ± 1.44), 6.64 (SD ± 1.03), and 6.96 (SD ± 1.32) for patients at cardiology clinics, FM clinics and AC clinic, respectively.

Conclusion: Based on the preliminary data the overall adherence to dabigatran or rivaroxaban in our patients was good with no significant differences between the clinical settings. Refill data is probably overestimating adherence and should be combined with another method of adherence estimation.

Disclosure of Interest: None declared.

PO356-TUE

A qualitative point of care test to assess anticoagulant activity in urine samples of patients treated with non-vitamin K antagonist oral anticoagulants – a german consensus

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Background: Rapid assessments of the anticoagulant effect of non-vitamin K antagonist oral anticoagulants (NOAC) by point of care testing (POCT) in specific patient populations and under certain clinical circumstances is an unmet clinical need. A POCT was developed detecting apixaban (A), rivaroxaban (R), and dabigatran (D) in urine samples through development of specific colors in the presence of NOAC which can be identified by naked eye within 10 to 15 min.

Aims: To develop consensus among a German group of experts to foster further evaluation of the POCT.

Methods: Two studies were used to develop the consensus: 1) The POCT were prepared in one center from patients treated with R and D (each $n = 15$) and of controls ($n = 15$) and were judged by naked eye by 30 participants to calculate sensitivities and specificities of the tests. 2) An international multicentre study was performed at 13 centers using 6 urine samples from patients on therapy with A, R, and D and 4 control samples. Kappa index, sensitivity, specificity, accuracy, negative and positive predictive values of the PCT tests were determined.

Results: From the first study, sensitivity and specificity of the POCT were both >0.98 for R and D. From the second study, kappa-index, sensitivity, specificity, accuracy, negative and positive predictive values were all >0.98 (95% confidence intervals between 0.89 and 1.00) for A,

R, and D. Heparins do not influence the results but macrohematuria does affect the POCT.

Conclusion: NOAC can be detected by POCT and the group of authors agrees that the POCT from urine samples is a rapid, sensitive and specific tool with the potential to provide information on the presence of NOAC. Control of adherence to therapy and assessment in emergency situations such as before surgery, following bleeding and thromboembolism will be the ultimate place for POCT and confirmation in real world settings are needed. Modification of the POCT to expand its use in blood samples is required.

Disclosure of Interest: None declared.

PO357-TUE

Paradoxical enhancement of the intrinsic pathway-induced thrombin generation in human plasma by melagatran, a direct thrombin inhibitor, but not edoxaban, a direct factor Xa inhibitor

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Background: The blood coagulation cascade consists of two pathways, the tissue factor (TF)-dependent extrinsic pathway and the contact factor-dependent intrinsic pathway. We have previously shown that a direct thrombin inhibitor, melagatran, paradoxically increased TF-induced thrombin generation (TG) in thrombomodulin (TM)-containing human plasma *in vitro*. However, the effect of melagatran on the intrinsic pathway-induced TG remains to be investigated.

Aims: To investigate whether melagatran enhances the intrinsic pathway-induced TG and this enhancement is mediated by the thrombin-TM and protein C (PC) system.

Methods: Kaolin-induced TG in human plasma was assayed by the calibrated automated thrombography method as follows: 80 μ L plasma in the presence and absence of anticoagulants and 5 nM TM were mixed with 20 μ L of a mixture of 600 ng mL⁻¹ kaolin and 24 μ M phospholipids. After 10 min of preincubation at 37 °C, the reaction was started by adding 20 μ L of a mixture of 2.5 mM fluorogenic substrate and 100 mM CaCl₂. The fluorescence was measured every 20 s for 120 min.

Results: Melagatran at 150 and 300 nM increased the peak level of TG by a maximum of 2.40-fold at 300 nM in normal plasma in the presence of 5 nM TM. At 600 nM, melagatran did not enhance TG. In the absence of TM or in PC-deficient plasma, the paradoxical enhancement of TG by melagatran disappeared. A direct FXa inhibitor, edoxaban, and an antithrombin-dependent anticoagulant, unfractionated heparin (UFH), did not increase, but simply decreased TG under each condition.

Conclusion: Melagatran enhanced the intrinsic pathway-induced TG as well as the extrinsic pathway-induced TG in human plasma under the condition where PC system is active. In contrast, edoxaban and UFH showed concentration-dependent decrease of TG, but no enhancement. These results indicated that edoxaban and UFH may have a low risk of the paradoxical enhancement of TG by both the extrinsic and intrinsic pathway activation.

Disclosure of Interest: None declared.

PO358-TUE

Evaluation of anticoagulation with rivaroxaban by anti-Xa activity, thrombin generation and complex thrombin anti-thrombin levels in patients with new onset non valvular atrial fibrillation

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Background: atrial fibrillation confers a hypercoagulable state with an increased thrombin generation (TG) capacity and raised levels of thrombin-antithrombin complexes (TAT), which results in a high risk of stroke and thromboembolism.

Aims: to evaluate the anticoagulant effect of Rivaroxaban by anti-Xa activity and its correlation with TAT, TG and prothrombin time (PT) in patients with newly diagnosed non-valvular atrial fibrillation (NVAf).

Methods: prospective study in patients with indication for anticoagulation. Demographic variables, cardiovascular risk factors, and HAS-BLED, CHA₂DS₂VASc scores were recorded. Baseline samples were taken at 4 and 24 h after the administration of the drug and at 30 days. Rivaroxaban levels, anti-Xa activity, PT, TG and plasma levels of TAT, were determined. The results were analyzed by Fisher test, "t" test, Pearson correlation and ANOVA in SAS/STAT[®] program.

Results: Twenty subjects, age 76.3 $\pm$ 8.0 years, 60% female, 95% CHADS₂Vasc score $\geq$ 2. The anti-Xa activity correlated with Rivaroxaban plasma levels at 3 h ($r = 0.61$, $P = 0.008$), 24 h ($r = 0.85$, $P < 0.0001$) and 30 days ($r = 0.99$, $P < 0.0001$), PT at 3 h ($r = -0.86$, $P = 0.019$) and 30 days ($r = -0.63$, $P = 0.02$) and a sustained decrease in platelet free plasma TG 30 days of follow-up ($r = -0.74$, $P = 0.0038$). No correlation with TAT ($r = -0.02$, $P = 0.83$) was observed.

Conclusion: Rivaroxaban induced inhibition of the mild procoagulant state found in newly diagnosed non-valvular atrial fibrillation (NVAf) patients, which was maintained through 30 days of treatment. The plasma levels of the drug correlated with PT, anti-Xa activity and decreased TG.

Disclosure of Interest: None declared.

PO359-TUE

Low dose rivaroxaban for secondary prophylaxis after full anticoagulation in patients with venous thromboembolism

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Background: After standard full anticoagulation for patients with 1st episode of unprovoked venous thromboembolism (VTE), the optimal duration of anticoagulation should take into account their inherited or acquired prothrombotic conditions, bleeding risk and preference. Due to predictable pharmacokinetics of new anticoagulants allowing multiple dosing options, the AMPLIFY-EXT study confirmed the role of low dose apixaban as secondary (2°) prophylaxis. Yet, apixaban has only been licensed for VTE treatment since 2015, patients requiring 2° VTE prophylaxis had to resort to other licensed drugs in Canada.

Aims: To review the clinical outcomes of a patient cohort being treated with low dose rivaroxaban (RIV_{low})

Methods: In an academic thrombosis clinic, patients receiving 10 mg RIV for 2° prophylaxis of VTE were reviewed. Apart from demo-

graphic and medical data, patients' thrombotic and bleeding outcomes were analyzed.

Results: From 2013 to 2014, a total of 35 patients were on continuous RIV_{low} for the following indications: single unprovoked VTE ($n = 17$), multiple provoked VTE ($n = 3$), single provoked VTE with ongoing risks ($n = 6$), multiple unprovoked VTE but intolerant to full anticoagulation ($n = 7$) and known high risk thrombophilia with sudden death in an immediate family member ($n = 2$). After a median of 14 months (median 12.7, range 1.7–34.4), 1 patient (3%) with antiphospholipid syndrome (APLS) required discontinuation of prophylaxis because of severe menorrhagia despite recurrent VTE. All other 34 patients (97%) did not have any recurrence. Mild iron (Fe) deficiency was found in 3 more patients (8.5%) likely due to preexisting menorrhagia, which resolved after replacement.

Conclusion: This patient cohort sheds light onto the safety profile of RIV_{low} for 2° prophylaxis after VTE, although RIV_{low} may be insufficient for APLS patients. The high prevalence of new onset Fe deficiency should prompt future clinicians to monitor Fe status routinely in those receiving low dose, yet long term anticoagulants.

Disclosure of Interest: None declared.

PO360-TUE

Intra-individual variability in trough and peak dabigatran levels in "real-life" atrial fibrillation patients

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Background: In patients with non-valvular atrial fibrillation treatment with dabigatran in fixed doses without routine coagulation monitoring and dose adjustment is recommended. However, plasma concentrations achieved with each dose vary widely across the patient population. In certain clinical situations measurements of the dabigatran effect may be desirable and relevant.

Aims: To estimate the intra-patient variability in peak and trough dabigatran levels.

Methods: In this prospective study we included 44 patients (71 ± 7 years, 25 women) who stated treatment with dabigatran 150 mg (D150) or 110 mg (D110) twice daily. After informed consent was obtained, they contributed 170 trough and peak blood samples that were collected 2–4 and 6–8 weeks after dabigatran initiation. The study was approved by the Slovene Ethics Committee. Plasma levels of dabigatran were assessed by measuring dabigatran concentration with LC-MS/MS and with coagulation tests: activated partial thromboplastin time (aPTT), Hemoclot thrombin inhibitor assay (HTI) and ecarin chromogenic assay (ECT).

Results: Patients receiving D110 were older, had lower renal function and higher CHADS₂ score than those receiving D150, but had similar concentration in both trough and peak samples. Intra-individual variability varied less in trough than in peak samples when dabigatran levels were measured with LC-MS/MS (17.0 ± 13.6 vs. $26.6 \pm 19.2\%$, $P = 0.02$) or estimated by aPTT (4.5 ± 3.3 vs. $9.5 \pm 6.5\%$, $P < 0.01$). Variability in trough and peak values measured by HTI (23.3 ± 20.4 vs. $25.7 \pm 19.2\%$, $P = 0.57$) and ECAT (20.9 ± 20.7 vs. $28.5 \pm 21.3\%$, $P = 0.13$) was not significantly different.

Conclusion: A low intra-individual variability of dabigatran concentration in trough samples suggests that one measurement may be appropriate to determine reliably trough dabigatran levels. Variability assessed by coagulation tests and in peak samples was considerably higher.

Disclosure of Interest: None declared.

PO361-TUE

Direct-acting oral anticoagulants in the real world: insights into health care providers' understanding of medication dosing and use

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Background: Direct-acting oral anticoagulant (DOAC) use is increasing in Canada. All DOACs require dose adjustment for renal function, and in some cases, for age as well. Manufacturers also provide instructions on DOAC administration. Dabigatran should not be exposed to moisture, nor should it be crushed. Higher doses of rivaroxaban should be taken with meals. Ideally, health care providers (HCPs) should counsel patients about appropriate DOAC use, but their understanding of these issues is unknown.

Aims: To assess nurse, pharmacist, staff physician and resident physician understanding of DOAC dosing and administration.

Methods: An electronic survey was distributed to HCPs at our tertiary care hospital, with questions on DOAC administration, dose adjustment, patient counselling and comfort level with DOACs.

Results: Fifty-nine responses were received – 10 from nurses, 1 from a pharmacist, 23 from staff physicians (hematology, general medicine, neurology), and 25 from residents. Only 10% of respondents correctly specified the approved indications for all three DOACs available in Canada. 32% felt comfortable or very comfortable prescribing DOACs. When counselling, 95% discussed bleeding, 47% discussed adherence strategies, and 64% discussed when/how to take the drug. 31% knew dabigatran should not be exposed to moisture, 52% that it should not be crushed. 29% knew higher dose rivaroxaban should be taken with food. 81%, 69% and 76% adjusted dabigatran, rivaroxaban and apixaban, respectively, for renal function. However only 61% calculated renal function with an accepted formula. (The remainder used laboratory-reported eGFR or creatinine alone.) 61% and 54% adjusted dabigatran and apixaban, respectively, for age.

Conclusion: HCP understanding of DOACs is variable. Though they express comfort with DOACs, their self-reported knowledge of dosing, administration and patient counselling is incomplete. HCPs have a unique opportunity to improve patients' medication literacy; strategies are needed to improve their knowledge base.

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PO362-TUE

Direct oral anticoagulants in clinical practice – real world audit data

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Background: The Anticoagulant Service at GWH currently provides counselling and dose adjustment of all patients commencing vitamin K antagonists (VKAs), discharge planning for inpatients and a telephone advice line for patients and all healthcare professionals. In June 2012 we adopted the UK NICE-TA for dabigatran and rivaroxaban for treatment of non-valvular AF in stroke prevention (SPAF) and in September 2013 apixaban was included.

Aims: Agreement was made with the local commissioners to continue to counsel patients commenced on the Direct oral anticoagulants (DOACs) with the aim of reviewing every patient to detect any adverse event.

Methods: Use of a decision aid ensured suitability of any proposed anticoagulant including checking for contra-indications, specialist groups where advice is required before prescribing and risk factors for

bleeding (HAS-BLED) and a proforma was developed for monitoring these patients. Patients are reviewed at 3 months and annually thereafter to check for any problems including bleeding, deteriorating renal function, episodes of dyspepsia or any other adverse event. Data is also collated for patients on a DOAC for treatment of VTE.

Results: Four-hundred and twenty-two patients receiving a DOAC for AF (10.5% of our total anticoagulant population) 66 on dabigatran of which 63 have had at least 1 review with 17 (27%) having an adverse event (2 major bleeds).

203 on rivaroxaban of which 179 have had a review with 25 (14%) having an adverse event (2 major).

153 on apixaban of which 131 have had a review with 3 (2.2%) having an adverse event (1 major).

Conclusion: In our patients' experience the DOACs appear to be well tolerated. All adverse events ranged from 2.2% to 27% but serious adverse events are low and similar ranging from 0.6–1.6%. Previous local adverse event data for patients receiving VKAs showed there was a 6% chance of an adverse event with a 1% risk of a major/fatal bleed and a 3% risk of a minor bleed. No patient has had an embolic event.

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PO363-TUE

Nuances of NOAC'S in a developing country – a single center experience

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Background: Stroke is a dreaded complication of atrial fibrillation (AF) and oral anticoagulants are effective in reducing the risk of stroke. New oral anticoagulants (NOAC's) which have advantages over vitamin K antagonists (VKA) are available now as alternatives.

Aims: 1) To study the prevalence of valvar and non-valvar AF (NVAF) in patients with AF 2) To assess the risk of stroke and bleeding in patients with NVAF and, 3) To study the frequency of usage of VKA and NOACs and their attendant problems.

Methods: All consecutively hospitalized patients with AF and those patients with AF seen in cardiology OPD were included in this observational prospective study. CHA2DS2-VASc score was used to assess the risk of stroke in patients with NVAF, and HASBLED score was applied to estimate the risk of bleeding. Renal function was assessed by Cockcroft formula.

Results: of 86 patients, 43 were male. The mean age was 66.9 years. Twenty-four patients had valvar AF, and 62 NVAF. 11 (12.7%) patients had stroke. 42 (48.8%) had hypertension, 18 (20.9%) had diabetes, 18 (20.9%) had coronary artery disease (CAD), 3 (3.4%) had cardiomyopathy, 17 (19.7%) were detected to have thyroid dysfunction and 7 (8.1%) chronic kidney disease (CKD) The mean CHA2DS2-VASc score was 3.0, and HASBLED score was 1.96.

All 24 patients of valvar AF received VKA. Out of 62 patients with NVAF, 15 received NOAC, 27 were on VKA (23 refused NOAC due to cost constraint and 3 had creatinine clearance $<30 \text{ mL min}^{-1}$) and 20 on antiplatelets (CHADS score 1 or less in 5 and 15 unwilling to take anticoagulant). Three switched to NOAC from VKA, and one opted to switch from NOAC to VKA. Of 51 patients who were on VKA, 2 had hemorrhagic stroke. Five patients died. No adverse effects were reported with NOACs.

Conclusion: 1. NVAF was more frequent than valvar AF. 2. Antiplatelets are still being used inappropriately 3. NOACs are attractive alternatives to VKA. 4. Cost is a major constraint in our practice for using NOACs.

Disclosure of Interest: S. Iyengar Speaker Bureau of: ASTRA ZENECA, PFIZER, Boehringer, R. Ravibabu: None declared, S. Lakshman: None declared, A. Shenoy Speaker Bureau of: ASTRA ZENECA, S. G: None declared, M. Fulmali: None declared, S. C. V: None declared.

PO364-TUE

Rivaroxaban pharmacokinetics before and after bariatric surgery: a case and discussion

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Background: Morbidly obese patients represent a special population with higher incidence of venous thromboembolism and atrial fibrillation (AF). As such, they may be considered for therapy with direct acting oral anticoagulants. Unfortunately, limited information is available regarding the pharmacokinetics (PK) of these agents in this group as patients with significantly elevated BMI were excluded or under-represented in clinical trials of these agents. The bioavailability of rivaroxaban (Riva) is particularly sensitive to food intake, with as much as a 40% increase in absorption in the fed compared to fasted state. When an obese patient undergoes bariatric surgery, they undergo physical alteration of their upper gastrointestinal tract, and an alteration in food intake, from fed to fasting. Despite a single published report of a patient who had bariatric surgery and then received Riva sometime after the surgery^{1,2}, it remains unclear what the impact of bariatric surgery is on the PK of Riva.

Aims: The aim of this report is to define the effects of bariatric surgery on the PK of Riva pre- and post-procedure.

Methods: Our anticoagulation clinic services patients with atrial fibrillation in the Southwestern Ontario region. Following appropriate informed consent, a 45 year old patient with AF and a BMI of 60, provided plasma samples for PK analysis.

Results: Pre-bariatric surgery, with BMI of 62 kg m^{-2} , the plasma concentration 19 h after Riva 20 mg was 9.2 ng mL^{-1} . This value falls below the 5th percentile (5th–95th percentile: 11–182). Six weeks after sleeve gastrectomy, weight decreased to BMI 53.5. Repeat concentration 1.8 h after Riva was 231.9 ng mL^{-1} . This value falls close to the expected mean concentration (Mean, 5th–95th percentile: 273.5, 184.3–398.5).

Conclusion: In this case, bioavailability of rivaroxaban increased after bariatric surgery. Available data on Riva concentrations versus BMI will be presented. The utility of a clinical pharmacology approach will be discussed.

Disclosure of Interest: G. Dresser Speaker Bureau of: Bayer, M. Gulilat: None declared, R. Kim: None declared.

PO365-TUE

Renal function course in patients treated with new oral anticoagulants

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Background: New Oral Anticoagulants (NOACs) may be prescribed for preventing ischemic stroke and systemic embolism in patients with non-valvular Atrial Fibrillation (AF), and for treatment and prophylaxis of venous thromboembolism. NOACs are characterized by a rapid onset of action, prevalent renal excretion, and a more predictable pharmacokinetics than Vitamin K Antagonists. Follow-up visits

must be scheduled to carefully assess both adherence to therapy and renal function, whose deterioration may be associated with increased bleeding risk.

Aims: To assess the course of renal function after starting NOACs.

Methods: Blood samples were collected before and after 1-month of NOACs treatment.

Results: Study population included 108 patients (54 females, 54 males, median age 77 years), referred to Atherothrombotic Unit of Careggi University Hospital, Florence, Italy. The average of baseline Glomerular Filtration Rate, estimated by Cockcroft-Gault formula (eGFR), was 64.5 (IQR 53.0–80.8) mL min⁻¹. In the overall population, no significant difference was observed between baseline and 1-month eGFR. By considering the indication for NOACs, in patients with AF, females showed a significant worsening of eGFR after 1-month [baseline: 63.0 (IQR 52.8–76.5) vs. 1-month: 57.3 (IQR 50.5–76.8) mL min⁻¹, $P = 0.048$]. No significant correlation was found between variation of eGFR [(baseline eGFR–1 month eGFR)/baseline eGFR] and CHA2DS2VASc score ($r = 0.168$, $P = 0.138$).

Conclusion: A worsening of renal function was observed in AF females on NOACs. These preliminary data need to be confirmed in a larger study addressed to a better stratification of the risk of renal function deterioration.

Disclosure of Interest: None declared.

PO366-TUE

The effects of direct oral anticoagulants apixaban and rivaroxaban on haemostasis tests: results from a comprehensive cross-laboratory exercise

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Background: The direct inhibitors of activated Factor X, apixaban and rivaroxaban are increasingly used for VTE prevention and therapy. They do not usually require laboratory monitoring but may interact with standard haemostasis tests. Testing to determine the degree of anticoagulation is useful in specific situations.

Aims: To determine the effects of apixaban and rivaroxaban on a wide range of haemostasis tests, primarily in Australasian laboratories.

Methods: Sixteen samples of pooled normal plasma spiked with apixaban or rivaroxaban, at levels covering the therapeutic range and beyond, were sent to 49 RCPAQAP Haemostasis participants. They were asked to perform a range of haemostasis tests.

Results: 90% of participants returned results. Rivaroxaban had more effect than apixaban on the APTT and prothrombin time; the sensitivity varied with different reagents. APTT and PT were largely unaffected by therapeutic levels of apixaban. One stage APTT factor assays (e.g. FVIII, FIX) showed false decreases with increasing concentration of apixaban or rivaroxaban. The Protein S clot-based assays exhibited a false increase in the level of expected PS with increasing levels of apixaban or rivaroxaban. However, the LIA free assays were unaffected by apixaban and rivaroxaban. Except for IL and Siemens Innovance assays (anti-Xa based) the antithrombin chromogenic assays were unaffected by apixaban or rivaroxaban. The dRVVT showed near linear prolongation with increasing apixaban or rivaroxaban. Good correlation was observed between expected levels of drug and participant results with anti-Xa assays. Thrombin generation tests were affected at all drug concentrations.

Conclusion: At most concentrations, these drugs affect the results of most haemostasis tests. Laboratories should become familiar with drug effects for correct interpretation of results. Our study provides useful data to assist in assessment of results in patients on apixaban or rivaroxaban.

Disclosure of Interest: None declared.

PO367-TUE

Effect of new oral anticoagulants on protein C system

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Background: Thromboembolism currently occupies the top three positions in the cardiovascular causes of morbidity and mortality in the developed world. In present time, novel oral anticoagulants, along with vitamin K antagonists, are widely used in clinical practice.

Aims: The aim of our study was to evaluate the effect of dabigatran and rivaroxaban on protein C (PC) system.

Methods: 86 patients with venous thromboembolism were studied, 17 on dabigatran, 69 on rivaroxaban. The control group consisted of 32 healthy persons. Thrombin generation was measured with Calibrated Automated Thrombogram using TF 5 pM. Endogenous thrombin potential (ETP, nM*min) was evaluated with and without addition of thrombomodulin (rh-TM). PC system efficacy was estimated by reduction of the ETP value (%) after addition of rh-TM. Statistica 6.0 package was used for the statistical analysis with nonparametric methods (median (Me), 95% confidence interval (95% CI) and Mann-Whitney U test were used for data description).

Results: Dabigatran patients' plasma revealed difference with control values, when rh-TM was added (+rh-TM: Me-1166.8, CI: 825.6–1600.0 vs. Me-878.0, CI: 538.8–1381.0, $P < 0.006$; –rh-TM: Me-1618.0, CI: 978.4–2099.0 vs. Me-1756.0, CI: 1220.6–2159.9, $P = 1.56$). ETP differed significantly from control in patients received rivaroxaban with or without rh-TM (Me-217.3, CI: 66.4–875.8 vs. Me-878.0, CI: 538.8–1381.0, $P < 0.0001$; Me-1062.1, CI: 571.8–1840.1 vs. Me-1756.0, CI: 1220.6–2159.9, $P < 0.0001$, respectively). We discovered the decrease of ETP values in patients on dabigatran or rivaroxaban vs. control after addition of rh-TM: Me-22.3, CI: 9.2–39.3 vs. Me-51.5, CI: 22.9–64.4, $P < 0.0001$; Me-79.5, CI: 36.4–89.2 vs. Me-51.5, CI: 22.9–64.4, $P < 0.0001$, accordingly.

Conclusion: These results suggest the enhancement of PC system in patients received rivaroxaban.

Disclosure of Interest: None declared.

PO368-TUE

A study of DOAC awareness indicating inadequate recognition with potential for patient harm

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Background: Many medical practitioners are unable to accurately indicate whether a patient on a DOAC is anticoagulated or not. This has serious implications for clinical management.

Aims: We assessed whether clinicians recognised from routine clinical information that the patient was anticoagulated and secondly whether it was safe to perform an invasive procedure.

Methods: In an on line study respondents were asked about their awareness of named DOACs. Then a clinical scenario was presented alongside coagulation test results and respondents were asked (i) is the patient adequately anticoagulated? (ii) is it safe to perform an invasive procedure on the patient.

Results: 143 individuals responded, 41% from primary and 59% from secondary care. 126 (88%), 115 (80%) and 72 (50%) recognised rivaroxaban, dabigatran and apixaban to be oral anticoagulants. Significantly more respondents in primary than secondary care recognised dabigatran ($P = 0.01$) but this was not so for rivaroxaban or apixaban ($P = 0.09$ and 0.1). Within secondary care, those in medical specialties were significantly more likely than others to be aware of dabigatran ($P = 0.008$) and apixaban ($P = 0.001$) but not rivaroxaban ($P = 0.11$).

Based on information that the patient had received a dose of anticoagulant 12 h before testing <17.5% knew the patient would be anticoagulated. Although a higher proportion recognised that it was not safe to perform an invasive procedure, almost 60% did not. Significantly more in secondary than primary indicated that it was not safe to proceed for rivaroxaban ($P < 0.035$) and dabigatran ($P < 0.005$) but there was no difference for apixaban. In secondary care those in medical specialties were significantly more likely to recognise the inadvisability of an invasive procedure in relation to dabigatran ($P = 0.003$) and apixaban ($P = 0.004$) but not rivaroxaban ($P = 0.2$).

Conclusion: Currently, recognition of DOACs and their management before invasive procedures are inadequate, with a high risk of patient harm. Improved training is required urgently.

Disclosure of Interest: None declared.

PO369-TUE

Circulating concentration of rivaroxaban or dabigatran anticoagulants in clinically selected patients

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Background: In accordance with guidelines patients receiving anticoagulants Rivaroxaban or Dabigatran do not require regular monitoring of circulating inhibitory levels. However, there are clinical situation where assessment of anti-Xa or anti-IIa activity may be appropriate to determine adequacy of anticoagulant effect or assessment in bleeding complications, particularly in patients prescribed potentially interacting medication or suffering from pathologies predisposing to anticoagulant complications.

Aims: To measure circulating Rivaroxaban or Dabigatran concentration in clinically selected patients to assess presence of anti-Xa or anti-IIa inhibition at 2–3 h post dose and correlate with reported Peak ranges.

Methods: Assays were performed on Destiny Max coagulation analyser (Stago UK), using patient citrated plasma. Dabigatran concentration was determined using Biophen DTI chromogenic anti-IIa assay and calibrated using specific calibrators and Controls (Hyphen BioMed). Rivaroxaban concentration was determined using Chromogenic Liquid Anti-Xa assay (Stago UK) and calibrated using specific calibrators and controls (Hyphen BioMed).

Results: Concentration of Rivaroxaban ranged from 70 to 475 ng mL⁻¹ with 23% of patients under and 29% over the Peak range of 175–360 ng mL⁻¹. for Dabigatran concentration ranged from 54 to 811 ng mL⁻¹ with 10% of patients under and 7% over the Peak range of 64–443 ng mL⁻¹.

Conclusion: Results found a number of patients had concentration of Rivaroxaban or Dabigatran outside Peak range which in association with interacting medication or other medical conditions may be clinically significant. However, trough level concentrations may be more clinically informative identifying adequate or inadequate anticoagulant cover between doses for thrombosis prevention and highlighting bleeding risk with increased levels.

Disclosure of Interest: None declared.

PO370-TUE

Comparing reversal effect of factor X concentrate and by-passing agents on rivaroxaban in an ex vivo spiking study with rotem

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Background: Despite the short half-life of the direct oral Xa-inhibitors immediate reversal of anticoagulant effect might still be required in the event of major bleeding or emergency surgery. Whilst both recombinant factor VIIa (rFVIIa) and prothrombin complex concentrate (PCC) act as by-passing agents, the use of FX concentrate (FX) would be a replacement strategy.

Aims: In this *ex vivo* spiking study, using blood samples from patients on rivaroxaban, we are comparing the reversal effect of FX, rFVIIa and PCC measured by rotational thromboelastography (ROTEM).

Methods: Citrated venous whole blood with and without CTI (20 µg mL⁻¹) was collected from 26 consecutive patients with atrial fibrillation on treatment dose Rivaroxaban. Rivaroxaban levels were measured in platelet poor plasma using anti-Xa assay (IL, USA) against rivaroxaban calibrators (Hyphen BioMed, France). for ROTEM analysis citrated whole blood was activated with a 1:50,000 dilution of Innovin® (Siemens, Germany) and 0.2 M CaCl₂ after *ex vivo* spiking with three different doses of FX concentrate (*in vivo* equivalent (IVE) 40, 20, 10 iu kg⁻¹), and two different doses for rFVIIa (IVE 100, 60 µg kg⁻¹) and PCC (IVE 40, 20 iu kg⁻¹).

Results: The prolongation of ROTEM parameters observed, was dependent on rivaroxaban plasma concentration which varied from 160 to 556 ng mL⁻¹ (mean 311 ng mL⁻¹). In response to *ex vivo* spiking dose-dependent percentage correction of baseline was observed with all three agents. At the highest dose of each agent used, percentage correction for clotting time was 25%(FX), 45%(rFVIIa), 45%(PCC), clot formation time 31%(FX), 66%(rFVIIa), 45%(PCC), maximum velocity 65%(FX), 157%(rFVIIa), 65%(PCC), time to maximum velocity 33%(FX), 51%(rFVIIa), 45%(PCC).

Conclusion: Substantive percentage reversal of ROTEM parameters was observed with all three agents. The safety profile of factor X concentrate could support higher *in-vivo* doses, for further correction of these ROTEM parameters. Efficacy will have to be tested further in clinical studies.

Disclosure of Interest: A. Drebes Grant/Research Support from: BPL; Bayer, A. Riddell: None declared.

PO371-TUE

Management of bleeding and emergency situations for patients treated with dabigatran- single centre experience

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Background: Dabigatran, a new oral anticoagulant with many benefits versus warfarin, has been increasingly prescribed for prophylaxis and treatment of thrombosis without age limitations. However, in the absence of an antidote the bleeding control and surgery remain to be a major challenge.

Aims: Management of surgery and major bleeds in patients treated with dabigatran admitted to our hospital in 2013–2014.

Methods: Five pats (84–93 years) with atrial fibrillation and satisfactory renal function at the start of dabigatran therapy (2 × 110 mg) developed serious clinical situations: 2 pats femurs fracture requiring

surgery and 3 pats major bleeds (soft tissue of the neck 1, gastrointestinal 1, retroperitoneal 1). We monitored INR, aPTTRatio (aPTTR), thrombin clotting time (TCT) and hemoclot thrombin inhibitors (HTI) and evaluated laboratory and clinical effect of prothrombin complex concentrate (PCC) and rFVIIa, administered in 3 and 1 pat, respectively.

Results: At admission (10–12 h after last dabigatran use) TCT was >150s and the range of aPTTR 2.8–3.8, INR 1.4–3.2 and HTI 200–370 ng mL⁻¹. All patients had reduced renal function (creatinine clearance <50 mL min⁻¹). PCC (20–40 IU kg⁻¹) resulted in a transient decrease of INR (1.1–1.4) with no influence on the aPTTR and TCT, which remained unchanged 48–96 h. Three pats survived thanks to a general supportive therapy. Two pats died due to the sequelae of bleeding. Patient 5 with massive retroperitoneal hematoma received PCC and single dose of rFVIIa (100 µg kg⁻¹) with no laboratory nor clinical effect and patient died due to hemorrhagic shock and heart failure.

Conclusion: Despite an excellent safety profile demonstrated in preregistration studies with dabigatran in all age groups, this medicament should be prescribed with a caution especially in elderly patients who, regardless to anticoagulant therapy have a higher probability of accidents as well as of the onset of comorbidity with renal function impairment.

Disclosure of Interest: None declared.

PO372-TUE

Bleeding and treatment failure in patients taking direct oral anticoagulants referred to emergency department: a cohort management study

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Background: Limited experience in the management of haemorrhagic and thromboembolic complications may determine the underuse of Direct Oral Anticoagulants (DOACs).

Aims: To report the results of a structured management of haemorrhagic and thromboembolic complications in patients treated with DOACs in the Emergency Department (ED) of a University Hospital.

Methods: We considered consecutive patients taking Dabigatran, Rivaroxaban or Apixaban referred to the Emergency Department for haemorrhagic or thromboembolic events.

Results: From January 2014 to January 2015, 32 patients reported bleeding complications (9 major and 23 minor bleedings using ISTH criteria) and 10 thromboembolic events. Intracranial haemorrhages (ICH) were 4, 3 of which were traumatic (subdural) and 1 was a spontaneous intraparenchymal bleeding. No one of the traumatic ICH required surgery and reversal of anticoagulation according to clinical judgement and drug concentration while the intraparenchymal bleeding was treated with 4-factors prothrombin complex concentrates (PCC, 50 U kg⁻¹). A pulmonary embolism followed by intestinal occlusion complicated the clinical course of this patient. Only 1 of the other major bleedings was treated with PCC and tranexamic acid: this was a traumatic upper limb bleeding in a patient taking a DOAC plus dual antiplatelet treatment. Two gastro-intestinal (GI) bleedings and 1 genital-urinary (GU) bleeding underwent blood transfusion. In only one patient with GI bleeding the drug concentration was high after 10 h from last intake: this was a 90 year old patient with renal impairment and low body weight. Among thromboembolic events, 7 were arterial (2 stroke, 4 TIA and 1 peripheral arterial embolism) and 3 were venous (2 PE and 1 DVT). In stroke patients aspirin 100 mg/die was added to treatment and DOAC was not changed in any case.

Conclusion: In this initial experience, patients treated with DOACs rarely needed the reversal of anticoagulation.

Disclosure of Interest: None declared.

PO373-TUE

Performances of a new commercial chromogenic assay for the measurement of apixaban concentration in plasma

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Background: Apixaban, an oral direct factor Xa inhibitor, is given at fixed daily doses and was cleared without the need of blood monitoring. However, the quantification of apixaban concentration in plasma can be needed in specific situations such as emergency surgery or during bleeding situation. A new chromogenic assay has been developed to allow rapid and automated testing to determine plasmatic apixaban concentration.

Aims: to assess the performances of this new commercial assay by comparison to a homemade ultra-performance liquid chromatography-tandem mass spectrometry method (LCMS).

Methods: 113 samples were tested, including 22 samples recruited on site and 7 spiked samples (for highest concentrations). Remaining samples came from clinical studies led by BMS (Bristol-Myers Squibb). The new assay for apixaban quantification in plasma was performed using STA[®] – Liquid anti-Xa with STA[®] – Apixaban Calibrator and STA[®] – Apixaban Control (Stago, Asnières s/Seine, France). The tests were run on a STA-R[®] analyser (Stago). The results were compared to LCMS performed on site, previously cross-validated with the LCMS reference method used by BMS.

Results: LCMS results ranged from 42 to 471 ng mL⁻¹. Correlation between the new chromogenic assay and LCMS was excellent ($r^2 = 0.981$) and more than 97% of results were within predefined criteria for Bland&Altman analysis. In addition, sample results from patients without apixaban confirm the good sensitivity around the quantification limit (23 ng mL⁻¹).

Conclusion: The comparison between the new chromogenic assay for apixaban and the LCMS method demonstrates the good overall correlation between both techniques. As this new assay will be easily available in haemostasis laboratory and is automatized, it will give to laboratories the possibility to quickly and safely measure apixaban concentration in plasma.

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PO374-TUE

Treatment options for major haemorrhage and urgent intervention in patients on a NOAC – interim results from the anticoagulation reversal and events study (ARES) collaborative

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Background: The ARES Collaborative is a large multicentre international observational prospective study of consecutive patients who present with haemorrhage, urgent surgery or thromboembolism who are taking non vitamin K antagonist oral anticoagulants; NOAC's – dabigatran, rivaroxaban, apixaban) or warfarin therapy. ACT Registry Number 12613001304729

Aims: Observe the choice and dose of haemostatic agent(s) used in NOAC patients who presented with major haemorrhage or for urgent reversal

Methods: Observational outcome data from the initial 38 NOAC patients who presented with major bleeding ($n = 27$) or requiring urgent surgery or intervention ($n = 11$).

Results: A majority of the patients were taking dabigatran (63.2%) with the remainder taking rivaroxaban (34.2%) and apixaban (2.6%). Major haemorrhage occurred primarily in the GI tract (44.4%) and CNS (37%). Prothrombinex VF was given in 16 patients at mean dose of 34.06 IU kg⁻¹ (range 20.3–47.6 IU kg⁻¹) and FEIBA in 6 patients at a mean dose of 45.4 IU kg⁻¹ (range 37.5–53.1 IU kg⁻¹). No FVIIa was utilised and almost half the patients did not received any haemostatic agent. Of the patients that received a haemostatic agent, 90% used only one dose and around half also received concomitant supportive care. >80% of patients presenting with NOAC related events had prolonged APTT/PT but only in 7/38 had specific drug assays. Overall 30 day mortality was 7.8%.

Conclusion: In NOAC patients with major haemorrhage, one dose of Prothrombinex VF or FEIBA (20.3–53.1 U kg⁻¹) appears to be sufficient if a haemostatic agent is used. Standard coagulation assays are prolonged in the majority of cases indicating the presence but not the drug level of the NOAC. Specific NOAC assays are rarely performed but should be encouraged to assist in the clinical decision process. The ARES collaborative continues to recruit 2000 patients with clinical adjudication of the haemostatic agent efficacy and assessment of NOAC drug assays to better inform clinical decisions for NOAC events.

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PO375-TUE

Prothrombinase induced clotting time (PiCT®) for measuring the activity of direct factor Xa inhibitors in clinical setting

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Background: To achieve efficient and safe anticoagulation with direct factor Xa inhibitors a close and reliable drug monitoring could be necessary in critical circumstances. Therefore is a need to find a universal test adaptable to standard instruments would simplify monitoring antithrombotic therapy with these drugs. Pefakit® PiCT® is a clotting assay sensitive to factor Xa and factor IIa inhibitors and could be used as a quantitative global test.

Aims: To evaluate the correlation between Pefakit® PiCT® and plasma levels of direct factor Xa inhibitors.

Methods: Using the Technoclone anti-Xa assay from Technoclone (Vienna-Austria), levels of direct factor Xa inhibitors were tested in 52 Blood samples from 26 patients, under treatment with rivaroxaban and apixaban, to determine the highest and the lowest blood levels of the drugs by obtaining a blood sample just before the morning dose then sampling the blood again 2 h after of the administered dose (calibration curves were obtained by measuring anti-FXa activity on different plasma calibrators with known amounts of every anticoagulant). Pefakit® PiCT® tests (Pentapharm, Basel-Switzerland) were performed in samples all blood samples, normal ratio for PiCT® assay was established by measuring clotting times on 86 normal samples.

Results: Clotting time ratio was prolonged in a concentration-dependent fashion at higher direct factor Xa inhibitors levels. There was a linear correlation between direct factor Xa inhibitors levels (ranging between 15.3 and 457.6 ng mL⁻¹) and ratios for Pefakit® PiCT® test. Pearson's correlation value was 0.72 with bilateral significance P value < 0.01.

Conclusion: Pefakit® PiCT® test, could be a valuable diagnostic test for rapid determination of the effect of direct factor Xa inhibitors and would allow therapeutical decisions in critical circumstances such as reversal of treatment or to ensure an optimal dose.

Disclosure of Interest: None declared.

PO376-TUE

An open-label study of dabigatran pharmacokinetics and pharmacodynamics in patients with moderate renal impairment undergoing primary unilateral elective total knee or hip replacement surgery

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Background: In patients undergoing total hip/knee replacement (THR/TKR), the recommended dose of dabigatran etexilate (DE) for prevention of venous thromboembolism is 220 mg once daily (qd). A lower dose (150 mg qd) is recommended for patients with moderate renal

impairment (RI; creatinine clearance (CrCL) ≥ 30 – ≤ 50 mL min⁻¹), but experience in these patients is limited.

Aims: To assess PK, PD and safety of DE 150 mg qd in patients with moderate RI undergoing THR/TKR.

Methods: A single-arm, open-label multicenter trial including adult, Caucasian patients with moderate RI. Patients received DE 75 mg 1–4 h after surgery and 150 mg qd from day 2–10 (TKR) or day 2–35 (THR), respectively. Steady-state blood sampling was at predose and 1, 2, 4, 8 and 24 h postdose on day 6 $\pm$ 1. Total plasma dabigatran concentrations were determined by HPLC with tandem mass spectrometry. PD parameters were dilute thrombin time, activated partial thromboplastin time and ECT. Ethics committee approval and informed consent were obtained ahead of the studies.

Results: 112 patients received DE (52 THR, 60 TKR; mean CrCL 42.5 mL min⁻¹; 69.6% female; mean age 79.1 years). Geometric mean (gMean) dabigatran trough plasma concentration predose ($C_{pre,ss}$) was 47.5 ng mL⁻¹ (interquartile range [IQR] 29.6–71.2). $C_{pre,ss}$ was comparable to that in patients with mild RI (CrCL 50– <80 mL min⁻¹) receiving DE 220 mg qd in RE-NOVATE II (30.2 ng mL⁻¹; IQR 19.4–55.0); a higher average concentration may reflect the older population with more females than in the phase III trial. Median time to gMean peak concentration (166 ng mL⁻¹) was 2.2 h. There were no thromboembolic events. Major bleeding occurred in 4 patients (2 surgical site and 2 gastrointestinal tract). PD results will be presented at the meeting.

Conclusion: DE 150 mg qd is an appropriate dose for patients with moderate RI undergoing THR or TKR. The rate of major bleeding is in the range expected without adjudication of events, given the small sample size.

Disclosure of Interest: B. Eriksson Consultant for: Boehringer Ingelheim, Z. Mikuska Employee of: Boehringer Ingelheim RCV GmbH & Co KG, M. Feuring Employee of: Boehringer Ingelheim Pharma GmbH & Co KG, J. Amiral Consultant for: Boehringer Ingelheim, S. Haertter Employee of: Boehringer Ingelheim Pharma GmbH & Co KG, J. Stangier Employee of: Boehringer Ingelheim Pharma GmbH & Co KG, G. Nehmiz Employee of: Boehringer Ingelheim Pharma GmbH & Co KG, M. Lobmeyer Employee of: Boehringer Ingelheim at the time of study conduct and analysis, J. Weitz Consultant for: Boehringer Ingelheim.

PO377-TUE

Qualitative follow up of emergency department (ED) patients discharged on rivaroxaban for low risk venous thromboembolism

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Background: FDA approval of rivaroxaban for venous thromboembolism (VTE) treatment has introduced another outpatient option from the Emergency Department (ED).

Aims: Qualitatively measure performance of rivaroxaban for ED VTE treatment.

Methods: Electronic health records identified ED patients discharged on rivaroxaban for treatment of VTE over 10-months. ED patients were contacted to determine if they were able to obtain and continue rivaroxaban and if dosing was appropriate. Patients were interviewed to determine their understanding of drug purpose and complications. Patients admitted or receiving rivaroxaban for atrial fibrillation were excluded.

Results: Out of 253 ED patients (95 discharged, 158 admitted) with an acute VTE diagnosis, 19 patients were discharged on rivaroxaban. The biggest barrier to not getting rivaroxaban prescribed was lack of approving insurance status at the time of ED encounter. Patient insur-

ance status was as follows: 11/19 patients had third party insurance, 3/19 Medicaid, 3/19 Medicare and 2/19 workman's compensation. 7/19 were not able to continue rivaroxaban due to financial reasons. 14/19 patients were able to be contacted by telephone and all stated they had received drug education and warned of potential risks. 2/14 patients reported that their dose was not correctly changed at day 21 or that their primary provider was not familiar with the drug or dose change. Chart review for the remaining 5/19 patients showed 1/19 had bleeding and 1/19 had a new PE while on rivaroxaban, each requiring subsequent hospital admission. 9/19 reported satisfaction with rivaroxaban.

Conclusion: The incorporation of insurance-approved rivaroxaban into a ED VTE Care Pathway appeared to be satisfactory in patients able to continue rivaroxaban. However appropriate follow up is still required as some patients experience complications. Further primary provider education is warranted and additional data is required to further clarify the benefits and risks of using rivaroxaban in this setting.

Disclosure of Interest: K. Nordenholz Grant/Research Support from: Boehringer Ingelheim, Consultant for: Roche, E. Thompson: None declared, T. Trujillo Consultant for: Janssen, G. Misky: None declared.

PO378-TUE

Net clinical benefit of dabigatran versus warfarin in prevention of recurrent venous thromboembolism: a pooled analysis of re-cover and re-cover II

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Background: In the RE-COVER/RE-COVER II trials in patients with acute venous thromboembolism (VTE), a fixed dose of dabigatran e-textilate (DE) was as effective as warfarin for prevention of recurrent VTE and was associated with a lower risk of bleeding.

Aims: To place the combined efficacy and safety of each anticoagulant in perspective, and to provide a more refined comparison of DE versus warfarin, we have calculated the net clinical benefit (NCB) of DE and of warfarin.

Methods: In both studies, patients were randomized to warfarin or warfarin-placebo plus parenteral anticoagulation for ≥ 5 days until international normalized ratio (INR) was ≥ 2 at 2 consecutive measurements. Parenteral therapy was then discontinued, and patients continued warfarin (target INR range 2.0–3.0) or received DE 150 mg twice daily for 6 months (double-dummy treatment period). Outcomes were centrally adjudicated. NCB was the composite of cardiovascular endpoints (nonfatal events of recurrent VTE, MI, stroke, or systemic embolism), all-cause death, plus bleeding outcomes consisting of (a) only major bleeding events (MBEs; narrow definition) or (b) MBEs and clinically relevant nonmajor bleeding events (CRBEs; broad definition). All individual endpoints within the combined NCB endpoints were weighted equally. Ethics committee approval and informed consent were obtained ahead of the studies.

Results: The narrow NCB outcome, which did not include CRBEs, showed similar NCB between patients who received DE (155/2553/6.1%) and those receiving warfarin (152/2554/6.0%), hazard ratio (HR) (95% CI) versus warfarin: 1.02 (0.81, 1.27). The broad NCB outcome, which included CRBEs, occurred in significantly fewer patients

who received DE (252/2553/9.9%) versus warfarin (308/2554/12.1%), HR (95% CI) versus warfarin: 0.80 (0.68, 0.95).

Conclusion: In the assessment of NCB in the prevention of recurrent VTE in patients with acute VTE, DE was similar to or superior to warfarin, depending on how NCB was defined.

Disclosure of Interest: S. Schulman Grant/Research Support from: Boehringer Ingelheim, Baxter, Octapharma, H. Eriksson Consultant for: Boehringer Ingelheim, Pfizer, Bayer Healthcare, Leo Pharma, Bristol-Myers Squibb, A. Kakkar Grant/Research Support from: Sanofi Aventis, Pfizer, Eisai Inc, GSK, Bayer Healthcare, Boehringer Ingelheim, Consultant for: Sanofi Aventis, Pfizer, Eisai Inc, GSK, Bayer Healthcare, Boehringer Ingelheim, Daiichi Sankyo, Bristol-Myers Squibb, C. Kearon Consultant for: Boehringer Ingelheim, S. Schellong Consultant for: Bayer Healthcare, Boehringer Ingelheim, Glaxo-SmithKline, Sanofi, M. Feuring Employee of: Boehringer Ingelheim, S. Hantel Employee of: Boehringer Ingelheim, J. Kreutzer Employee of: Boehringer Ingelheim, S. Goldhaber Grant/Research Support from: Bristol-Myers Squibb, Boehringer Ingelheim, Consultant for: Boehringer Ingelheim, Merck, Bristol-Myers Squibb, Medscape.

PO379-TUE

Identification of possible drug related problems in patients on oral anticoagulants. A systematic review of medication charts by pharmacist

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Background: The use of oral anticoagulants is effective in many thrombotic diseases but carry significant risk for complications.

Aims: The purpose of this study was to identify and manage drug-related problems (DRPs) related to the treatment with oral anticoagulants in hospitalised patients.

Methods: 50 patients, above 18 years of age, from three internal medicine departments were included. The patients was on medication with or would start treatment with oral anticoagulants. Based on information from the medication chart, journal and laboratory values, each patient's drug regimen was then systematically reviewed by a pharmacist to identify DRPs. Identified DRPs with proposed solution was presented for the physician after the physician's visit. The physician's response and possible actions to manage the DRPs were registered.

Results: A total of 50 patients were included, 26 on warfarin (52%) and 24 patients on direct oral anticoagulants (DOAC). Overall, 62 DRPs were identified in 35 of the included patients (70%). Of these, 37 DRPs (1.42 DRPs per patient) were related to warfarin treatment and 25 DRPs (1.04 DRPs per patient) related to treatment with DOAC (difference not statistically significant). However, a significant difference was observed in the number of drug interactions in the warfarin group compared to the DOAC group, 1.35 and 0.54 interactions per patient ($P = 0.015$) respectively. The pharmacist discussed 19 of the 62 DRPs with the physician, of which 2 DRPs were related to warfarin and 17 DRPs to DOAC. Corresponding changes to the patient's medical treatment were performed in 11 cases for DOAC and none for warfarin.

Conclusion: This study indicates a lower incidence of DRPs among patients treated with DOAC compared to warfarin. Furthermore, the results indicate that DRPs recorded and reported by a pharmacist lead to significant changes in the patients' medical treatment. Systematic reviews of patients' medication by a pharmacist could be an important tool for optimal anticoagulation.

Disclosure of Interest: None declared.

PO380-TUE

Influence of DOACs on thrombin generation assay

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Background: The principle that new oral anticoagulants (DOACs) do not require laboratory monitoring is controversial by ongoing that laboratory testing for drug effects is needed in many situation.

Aims: In this study, we aimed to examine the effects of DOACs (dabigatran, rivaroxaban, and apixaban) on the thrombin generation assay (TGA) *in vivo*.

Methods: We administered 12 healthy volunteers regular doses of DOACs, and measured TGA before and after administration (2, 4, 6, 12, 24 h later). TGA measured it using Calibrated Automated Thrombogram (CAT) method. We compared four indexes such as lag time, peak height, area under curve (ETP), time to peak as a parameter of TGA.

Results: As a result, rivaroxaban and apixaban, which are direct Xa inhibitors, significantly decreased TGA after administration within 2–4 h. In apixaban, parameter of TGA, such as ETP and peak height after 12 h of taking the drug was significantly inhibited. Also in rivaroxaban, although there were milder effects as compared with apixaban, thrombin production such as ETP and peak height after 24 h of taking the drug was significantly decreased. On the other hand, with dabigatran, which is a direct thrombin inhibitor, a paradoxical effect was seen in the peak height and ETP, which are criteria to evaluate TGA after administration. However, a prolonged lag time and time to peak were observed in each time zone after administration, and similarly to rivaroxaban and apixaban, such prolongation was also observed during 24 h after the initiation of administration.

Conclusion: From these results, it is suggested that TGA is useful for assessing the coagulation status of DOACs. Rivaroxaban was significantly suppressed the TGA during the 24 h. Also, apixaban and dabigatran (lag time and time to peak) were significantly suppressed the TGA during the 12 h.

Disclosure of Interest: None declared.

Platelet activation / adhesion / aggregation II

PO381-TUE

New factor of platelet hemostasis pathology risk marker in population of nenets autonomous district

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Background: Subclinical inflammation processes play an important role in the pathogenesis of coronary heart disease and its acute form – myocardial infarction. In recent years it was proved that sCD40L expressed in platelets is a link between inflammation, atherosclerosis and thrombosis. In healthy individuals it is regarded as a predictor of future thrombotic complications risk.

Aims: The aim of the study is to investigate blood concentration of sCD40L, a cardiovascular pathology marker, in the indigenous Nenets autonomous district ethnoses.

Methods: The research design is cross-sectional study. Inclusion criteria were: the indigenous Nenets ethnicity, permanent residence in the Nenets autonomous district, voluntary informed consent presence. Collection of biological material and epidemiological data was performed during prophylactic medical examinations in 2011–2012 according to GCP. The research protocol was approved by local ethics committee (protocol №6, 8.06.2011). The number of participants was 68. The average age was $Me = 42$ [30;51]. Blood concentration of sCD40L was determined with Human Cardiovascular 6plex BMS811/

2FF kit at flow cytometer Cytomics FC 500. Numerical values are given as median (Me) and 25th and 75th percentiles.

Results: The sCD40L concentration in Nenets blood was Me = 7.77 [5.1; 13.2] ng mL⁻¹. Upper limit of normal reference is 1.5 ng/mL. Our results show increased platelets functional activity and, as a consequence, increased thrombosis risk.

Conclusion: Study of new cardiovascular pathology risk factor markers in Nenets population let us talk about serious violations of platelet hemostasis. Determination of sCD40L in blood may help to reveal people with increased cardiovascular disease risk before clinical manifestations and to perform its early prevention in this population.

Disclosure of Interest: None declared.

PO382-TUE

The effect of IgG of ischemic stroke patients on platelet proteins secretion

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Background: IgG is the main immunoglobulin subtype in the circulation and they have often been suggested to be proatherogenic. IgG can activate platelets causing secretion of coagulation mediators.

Aims: To determine IgG levels in ischemic stroke and analyze the effect of IgG on platelet proteins secretion.

Methods: The study included atherothrombotic and cardioembolic ischemic stroke patients and individuals without a history of stroke. All patients received aspirin 325 mg with subsequent daily dosage of 100 mg + low molecular weight heparins. IgG were separated by affinity chromatography. Donor platelets were obtained by gel filtration.

Results: In patients with cardioembolic ischemic stroke IgG level was reduced by 30% on admission and elevated by 40% on the 14-th day relative to donors. In atherothrombotic ischemic stroke IgG level was within the norm, decreasing by 20% on the 14-th day. IgG of all study groups induced secretion of HSP-60 and HSP-70. IgG of patients with cardioembolic ischemic stroke caused more intense HSPs secretion compared with IgG of 2 other groups. HSP-60 was elevated by 20% compared with atherothrombotics and controls and HS-70 by 27% and 13% respectively. IgG of stroke groups caused active secretion of von Willebrand factor (vWF). IgG of patients with cardioembolic ischemic stroke caused more intense (by 15%) vWF secretion compared with atherothrombotic ischemic stroke group. We did not detect secretion of plasminogen activators inhibitor-1 from platelets under influence of any IgGs.

Conclusion: IgG of stroke groups caused secretion of HSP-60, HSP-70 and vWF from platelets. IgG of patients with cardioembolic ischemic stroke provoked more intense protein secretion.

Disclosure of Interest: None declared.

PO383-TUE

Meal-induced platelet activation differs between patients with type 1 and type 2 diabetes and is related to insulin secretion

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Background: Postprandial platelet activation was related to postprandial insulin rather than glucose levels in a previous meal insulin study in type 2 diabetes mellitus (T2DM).

Aims: To compare postprandial platelet activation in type 1 (T1DM) patients without insulin secretion and T2DM patients with high postprandial insulin levels.

Methods: Patients with T1DM ($n = 11$) and T2DM ($n = 12$) were studied before and 90 minutes after a standardized meal without premeal insulin. Platelet activation stimulated by the thromboxane analogue U46619 or ADP was assessed by flow cytometry, and by whole blood aggregometry (WBA; Multiplate®). Five patients with T1DM were restudied after taking their regular premeal insulin (a pilot study), and effects of insulin (100 µU mL⁻¹) *in vitro* were also studied.

Results: Before the meal, glucose, insulin and platelet activation markers did not differ between T1DM and T2DM. Platelet-leukocyte aggregates (PLA) were higher in T1DM. Postprandial glucose levels increased more markedly in T1DM (to 22.1 ± 1.4 vs. 11.9 ± 0.6 mmol L⁻¹) while insulin levels increased in T2DM only (from 23.2 ± 5.7 to 71.2 ± 15.9 mU mL⁻¹). Platelet P-selectin expression and PLA formation stimulated by U46619 were approximately doubled and whole blood aggregation stimulated by U46619 was increased by $\approx 20\%$ ($P < 0.05$ for all) after the meal in T2DM patients without any meal effect in T1DM patients. *In vitro* insulin mildly activated platelets in both groups. Premeal insulin enhanced the postprandial U46619 stimulated platelet activation ($P < 0.05$ vs. without insulin by ANOVA) in T1DM similarly as previously found in T2DM.

Conclusion: When no insulin was given prior to the meal, postprandial platelet activation was found in T2DM but not in T1DM despite higher postprandial glucose levels in T1DM. Postprandial platelet activation in diabetes is related to postprandial insulin levels and not to postprandial hyperglycaemia.

Disclosure of Interest: None declared.

PO384-TUE

Influence of platelet count in platelet rich plasma for adenosine triphosphate release assay

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Background: Platelet dense granule release assays are recommended for diagnosing platelet function disorders and are commonly performed by bioluminescent assays that measure stored adenosine triphosphate (ATP) release in platelet rich plasma (PRP) using a lumi-aggregometer. *for* light transmission platelet aggregation (LTA) guidelines are available on the platelet count in PRP (between 150 and 600×10^9 L⁻¹). Equally, the ATP release assay may also be influenced by the platelet count in PRP, however, there are no recommendations.

Aims: We evaluated the influence of platelet count in PRP on the ATP release assay with 1 U mL⁻¹ thrombin, performed by lumi-aggregometry (Chrono-Log, Havertown PA, USA).

Methods: We performed dilution series of PRP (platelet count $50\text{--}625 \times 10^9$ L⁻¹) from 10 different volunteers and correlated with ATP release. Also, we correlated ATP release and platelet count in PRP retrospectively in patient with suspected bleeding diathesis with normal LTA and ATP release (≥ 0.5 nmoles) ($n = 70$).

Results: In the serial dilution samples a good correlation (linear regression $r^2 = [0.86\text{--}0.99]$) between ATP secretion and platelet count was observed. There was a broad inter-individual variability in cut-off for the platelet count ($195\text{--}547 \times 10^9$ L⁻¹) corresponding with ATP release > 0.5 nmoles.

The mean ATP secretion of the patient samples ($n = 70$) was 0.90 nmol (range 0.50–3.36 nmol) and a mean platelet count in PRP of 355×10^9 L⁻¹ (range $135\text{--}673 \times 10^9$ L⁻¹). The correlation in patient samples was lower but significant (Spearman's $r = 0.316$, $P = 0.008$). Linear regression analysis showed large inter-patient variability ($r^2 = 0.055$).

Conclusion: We observed a significant correlation between ATP release and platelet count in PRP. Our dilution series and the retrospective analysis showed that the effect of platelet count in PRP on the ATP release showed an inter-individual variability. Because the ATP release is dependent on the platelet count in PRP, guideline development might be helpful to improve practices for performing ATP release assays.

Disclosure of Interest: None declared.

PO385-TUE

Activation of human platelets by *Staphylococcus aureus* secreted protease staphopain A

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Background: *Staphylococcus aureus* is a major human pathogen that causes life threatening bacteremia, resulting in an increased risk of myocardial infarction or stroke and is also the leading aetiological agent of infective endocarditis (IE). The pathogen activates platelets causing aggregation and thrombus formation, which are regarded as important steps in the pathogenesis of IE. Staphopain A is a cysteine protease secreted by *S. aureus*.

Aims: To investigate the effect of Staphopain A on platelets.

Methods: Using light transmission aggregometry (LTA), supernatant from a staphopain A deficient *S. aureus* mutant was unable to activate platelets when compared to the parental strain. LTA determined the effect of staphopain A on platelets and specific inhibitors and antagonists elucidated its cognate receptor. Better understanding of the effects of staphopain A on thrombus size was achieved by studying their formation under flow. Integrin $\alpha_{IIb}\beta_3$ activation and P-selectin exposure on platelets activated by staphopain A were measured using flow cytometry.

Results: We demonstrate that staphopain A induced the aggregation of human platelets. The presence of staphopain A in the supernatant of *S. aureus* conferred an ability to activate platelets. Staphopain A was also found to induce the secretion of ATP and potentiate the action of thrombin and crosslinked collagen related peptide. Staphopain A platelet agonist activity was inhibited by addition of the specific inhibitor staphostatin A, implicating its protease activity in the agonism. Concentrations of staphopain A that were insufficient to induce platelet aggregation caused increased platelet binding to collagen. Using specific antagonists, protease-activated receptors 1 and 4 (PAR1, PAR4) were shown to be responsible for mediating staphopain A induced activation of platelets.

Conclusion: PAR1 and 4 are receptors for staphopain A. Staphopain A mediated activation of protease activating receptors represents a novel axis in the human host-*S. aureus* interaction.

Disclosure of Interest: None declared.

PO386-TUE

Low platelet reactivity is associated with more severe atherosclerosis and increased revascularization during follow up

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Background: Platelets play a central role in haemostasis and wound repair. It is commonly accepted that high platelet reactivity increases

the risk of acute ischaemic occlusion upon rupture of atherosclerotic plaques. The role of platelets in chronic atherosclerotic plaque formation has not been established in humans. Therefore, we have studied the relation between platelet reactivity and atherosclerotic plaque morphology in patients with coronary artery disease (CAD).

Aims: To investigate the role of platelet reactivity in patients with chronic atherosclerotic disease.

Methods: In 202 patients undergoing coronary angiography we measured platelet activation by assessing P-selectin expression and fibrinogen binding in response to stimulation of the major platelet activation pathways. Patients were assigned into three groups; low, normal and high platelet reactivity (LPR, NPR and HPR). Based on visual assessment of angiographic images, patients were classified into: no CAD, minor CAD and significant CAD. One- and two year follow up was collected by questionnaires. This study conforms to the declaration of Helsinki and was approved by the local medical ethical committee. All patients provided written informed consent.

Results: ST-elevated myocardial infarction (STEMI) as indication for the angiography, symptomatology of unstable AP (UAP, nSTEMI or STEMI) and increased angiographic significance of CAD was more common in LPR than in NPR or HPR ($P < 0.001$, $P < 0.001$ and $P < 0.001$). In two-year follow up no differences were found in death, but there was higher incidence of re-PCI in the LPR group than in the NPR and HPR (15.6%, 3.8% and 3.9%). These findings were not explained by usage of platelet inhibitors.

Conclusion: Low platelet reactivity is associated with more severe CAD and higher incidence of re-PCI during follow-up. This finding indicates that an adequate platelet function is required to control atherosclerotic plaque formation.

Disclosure of Interest: None declared.

PO387-TUE

Evaluation of Multiplate[®] whole blood impedance aggregometry in routine investigation of bleeding disorders

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Background: The Multiplate[®] platelet analyser (MEA) performs impedance aggregometry in whole blood providing rapid screening of platelet inhibition by aspirin and clopidogrel.

Aims: To evaluate its potential role in routine diagnosis of bleeding disorders by correlating with traditional platelet function testing.

Methods: All 86 patients studied had a history of bleeding and/or bruising; including von Willebrand disease (vWD) or suspected vWD (24), thrombocytopenia (10) or myeloproliferative disorder (6). Patients known to be on antiplatelet therapy at time of testing were excluded. MEA was performed with the agonists ADP, ADP with prostaglandin E, arachidonic acid, TRAP, collagen and high and low dose ristocetin. Other tests (performed on majority of patients) were light transmission platelet aggregation (LTA) on an AggRam aggregometer (assessed by maximal amplitude only), INNOVANCE[®] PFA-200 and vWD screen (performed by Pathology West).

Results: On MEA, 36 patients had abnormally low response to one or more agonists. 20 patients were found to have thrombocytopenia (mostly mild) and half of these had at least one defect (mostly ADP). 7 patients' results were consistent with aspirin ingestion. vWD screening identified 24 patients with vWD or borderline vWD. On MEA, only 1/4 with Type 1 vWD (3 moderate and 1 mild), 3/7 with Type 2 and 3/13 with borderline vWD had a reduced response to ristocetin whereas 15 of 21 patients tested had an elevated PFA-200 (both Epi and ADP). Interestingly, only one patient had an abnormal response to ristocetin by LTA. Two patients with vWD had MEA performed serially after infusion of desmopressin and Biostat[®]. Additionally amongst the 62

patients not identified as having vWD (or borderline), 15 had abnormal PFA-Epi (9 also with abnormal PFA-ADP) with 5 patients having no other abnormal platelet function tests.

Conclusion: The diagnosis of bleeding disorders, including vWD does not appear to be enhanced by addition of Multiplate® testing to existing assays.

Disclosure of Interest: None declared.

PO388-TUE

Analysis of procoagulant PS-exposing platelets by imaging flow cytometry

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Background: The multifunctional nature of platelets lends itself to a heterogeneous platelet response. Here, we focus on the procoagulant subpopulation of platelets that is formed upon activation and distinguished by the exposure of phosphatidylserine (PS) on the surface membrane.

Aims: To evaluate the physical characteristics of PS-exposing platelets.

Methods: Washed platelets were stimulated with 1 U mL⁻¹ thrombin and 10 µg mL⁻¹ collagen (T+C) or 3 µM A23187. Their ultrastructural features were examined by transmission electron microscopy (TEM) and the Amnis ImageStream imaging flow cytometer. In the latter, 150,000 cells were collected/sample, annexin A5 binding identified PS-exposing (PS+) platelets and image analysis features were determined with IDEAS software. Data presented are mean±SEM, *n* = 3–4 experiments.

Results: A subpopulation of the highly activated T+C-stimulated platelets and all A23187-stimulated platelets were observed by TEM to have a striking spherical, empty, 'balloon-like' morphology. Imaging flow cytometry revealed that these unique platelets were PS+. Comprehensive analysis demonstrated that the PS+ platelets were comparable in size to resting and T+C(PS-) platelets. However, they were significantly more spherical, as indicated by an increased 'circularity' score: 12.7 ± 0.51 [T+C(PS+)] and 13.2 ± 0.63 [A23187(PS+)] vs. 9.6 ± 0.18 [resting] and 9.2 ± 0.15 [T+C(PS-)]; *P* < 0.001. Additionally, they were dramatically lacking internal structures, as indicated by decreased dark field signals including 'intensity' and 'bright detail intensity' e.g., 'intensity' scores: 6125 ± 339 [T+C(PS+)] and 8.111 ± 1.103 [A23187(PS+)] vs. 21,729 ± 1799 [resting] and 23,209 ± 2461 [T+C(PS-)]; *P* < 0.001.

Conclusion: The innovative imaging flow cytometer allowed detailed fluorescence-based and morphometric analysis of T+C or A23187-activated PS-exposing platelets; in becoming procoagulant, platelets undergo remarkable morphological changes, transforming into spherical 'balloons', almost devoid of their normal internal architecture.

Disclosure of Interest: None declared.

PO389-TUE

The potential of the new platelet activation in unprocessed blood (PACT-UB)- test

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Background: P2Y12 inhibitors are prescribed to prevent secondary cardiovascular events. Although successful on population level, P2Y12 inhibitors induce either thrombosis or bleeding complications in many individual patients. This complication rate may be reduced by tailored adjustment of treatment intensity, based on platelet function monitoring. Several global haemostasis tests have been used for these purposes, but failed by lack of sensitivity or specificity.

Aims: The aim of the current study was to investigate the impact of P2Y12 inhibitors on different platelet activation responses, induced by multiple agonists.

Methods: We have developed, optimized and evaluated a novel sensitive multiplex Platelet Activation Test (PACT) to assess on-treatment platelet reactivity, based on quantification of alpha granule release and on activation of the α_{IIb}β₃ receptor.

Results: Using the PACT, we showed that ADP mediated platelet activation can be completely blocked with high dose P2Y12 inhibitors, while PAR1, PAR4 and GPVI induced fibrinogen-binding capacity is strongly inhibited. In contrast to fibrinogen-binding capacity, PAR1, PAR4 and GPVI induced P-selectin expression is not affected by P2Y12 inhibitors, indicating a differential activation pattern. All findings are confirmed *in vitro*, under flow and in blood of patients on clopidogrel treatment.

Conclusion: Secondary platelet activation via the P2Y12 receptor enhances fibrinogen binding to activated platelets upon PAR-1, PAR-4 and GPVI induced activation while it has minor impact on P-selectin expression. These findings indicate that P2Y12 inhibitors impair platelet thrombus formation, without affecting granule release. Our study provides new insights in on-treatment platelet function, which may be crucial for adjustment of treatment intensity with P2Y12 inhibitors.

Disclosure of Interest: None declared.

PO390-TUE

Determination of threshold agonist concentrations for whole blood aggregation using the Multiplate® analyzer

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Background: The Multiplate® analyzer (Diapharma) is an automated multi-channel whole blood aggregometer that measures platelet aggregation as a change in impedance across two pairs of electrodes in each test cell. Most published studies of the Multiplate® have used manufacturer-recommended concentrations of manufacturer-supplied reagents that result in maximal aggregation responses.

Aims: 1) To determine the optimal concentrations of agonists, defined as threshold concentrations producing ≥ 80% of maximum aggregation; 2) To compare inter-laboratory variability of results of testing.

Methods: Blood samples were collected in hirudin from healthy adult volunteers with no exposure to platelet-inhibiting drugs for at least

10 days. ADP, collagen, and arachidonic acid (Helena Laboratories), and thrombin receptor activating peptide (TRAP; Diapharma) were tested at recommended concentrations and at a series of lower concentrations. Results of parallel aggregation testing at two research laboratory sites were compared.

Results: Dose response curves were generated for each agonist. Threshold concentrations were significantly less than manufacturer-recommended concentrations for all agonists: ADP, 3.0 vs. 6.5 μM ; collagen, 1.0 vs. 3.2 $\mu\text{g mL}^{-1}$; arachidonic acid, 0.2 vs. 0.5 mM; TRAP, 10 vs. 32 μM . Significant inter-laboratory variations in aggregation response were identified for sub-threshold concentrations of ADP (0.8 μM ; $P < 0.01$), collagen (0.3 $\mu\text{g mL}^{-1}$; $P < 0.05$) and TRAP (7 μM ; $P < 0.001$), and for spontaneous aggregation ($P < 0.001$), but not for arachidonic acid (0.13 mM).

Conclusion: The threshold concentrations for all agonists were significantly lower than recommended by the manufacturer. Comparison of results from two laboratories using the same analyzer and reagents showed significant differences in response. These findings reinforce the requirement for laboratories to develop site-specific reference intervals for analyzer/reagent pairs and agonist concentrations for optimal testing.

Disclosure of Interest: S. Israels Grant/Research Support from: Novo Nordisk, E. McMillan-Ward: None declared, H. Wang: None declared, V. Blanchette Grant/Research Support from: Novo Nordisk, M. Rand Grant/Research Support from: Novo Nordisk.

PO391-TUE

Assessment of time-related preanalytical influences on platelet function: comparison of the multiplate, the PFA-100 and the verify now

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Background: Platelet function testing may help to monitor treatment efficacy in patients treated with antiplatelet drugs and to identify clopidogrel poor responders. Three commonly used point of care methods use ADP stimulation to monitor P2Y₁₂ inhibition in whole blood.

Aims: The aim of this study is to compare three platelet function test systems (Multiplate, PFA-100 and Verify Now) in clopidogrel or prasugrel treated patients and in healthy volunteers with special focus on the influence of time-delay on the tested parameters and the concordance between the three assays.

Methods: Nine healthy volunteers and thirty-six patients, who were either treated with clopidogrel or prasugrel, were included in the study. ADP induced platelet function was assessed 1 and 3 h after sampling using the Multiplate, PFA-100 and Verify Now.

Results: PFA-100 was highly sensitive to P2Y₁₂-inhibition and demonstrated non-closure time in 23 patients. A more graded response could be detected with the two other devices. Multiplate aggregation data and PFA-100 closure times of all participants correlated negatively ($R = -0.51$), whereas no correlation could be found between Multiplate and Verify Now. Aggregation levels measured with the Multiplate after one hour correlated strongly with aggregation levels after three hours ($R = 0.78$), however platelet aggregation was reduced at 3 h compared to 1 h, especially in healthy volunteers ($P < 0.05$). In contrast, Verify Now values did not reveal any difference in healthy volunteers between 1 and 3 h, whereas P2Y₁₂% inhibition values increased after three hours in Clopidogrel or Prasugrel treated patients ($P < 0.05$).

Conclusion: Aggregation responses are time-dependent after blood sampling for the Multiplate in healthy individuals and for the Verify Now in Clopidogrel or Prasugrel treated patients. As for both devices,

platelet aggregation was reduced at 3 h post sampling which may affect data interpretation and clinical consequences.

Disclosure of Interest: None declared.

PO392-TUE

Dynamics of calcium spiking, mitochondrial collapse and phosphatidylserine exposure in single platelets during activation

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Background: Activated platelets form two distinct subpopulations, one of which is characterized by high intracellular calcium concentration, loss of mitochondrial membrane potential and phosphatidylserine (PS) exposure. However, there is little information about detailed relationship between dynamics of these three processes.

Aims: To elucidate the sequence of events leading to PS exposure.

Methods: Washed gel-filtered platelets were loaded with FuraRed and tetramethylrhodamine, labeled with annexin V and immobilized on a fibrinogen coverslips, activated with TRAP or thrombin and registered using confocal microscopy in real time. For experimental planning and analysis, we used a computational model of PAR1-stimulated platelet signal transduction that described interactions between cytosol, dense tubular system and mitochondria. The set of ordinary differential equation was solved using COPASI software (www.copasi.org).

Results: TRAP stimulation induced spiking in cytosolic calcium that gradually disappeared as a result of PAR1 inactivation. In some platelets, spiking led to a significant accumulation of calcium in mitochondria via the mitochondrial uniporter. This resulted in a mitochondrial permeability transition pore opening, loss of mitochondrial membrane potential, and the PS exposure (all three processes taking place with no detectable time shift). Interestingly, the procoagulant platelets initially had significantly higher average calcium concentration in their resting state than those that did not expose PS, suggesting a possible cause for the difference in their subsequent fate.

Conclusion: Procoagulant platelets form as a result of mitochondria overloading with calcium following a prolonged period of cytosolic calcium spiking. Initial cytosolic calcium level in a resting platelet can pre-dispose it to becoming either procoagulant (if high) or non-procoagulant upon activation.

Disclosure of Interest: None declared.

PO393-TUE

GPVI and the CLEC-2/podoplanin axis as potential biomarkers of platelet activation in inflammatory conditions

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Background: Soluble Glycoprotein VI (sGPVI) is a recognised marker of platelet activation in inflammatory diseases. The closely related platelet signalling receptor, CLEC-2, and its ligand podoplanin, are also potential biomarkers of inflammation, expressed as soluble protein or on the surface of extracellular vesicles.

Aims: To compare soluble and platelet-derived GPVI and CLEC-2 and podoplanin as biomarkers of inflammation.

Methods: Sandwich enzyme-linked immunosorbent assays (ELISA) were used to measure sGPVI and sCLEC-2 in plasma samples from a longitudinal study of 38 burns patients and in patients with rheumatoid arthritis (RA). The results were compared to measurements of GPVI, CLEC-2 and podoplanin in microvesicles.

Results: Measurements were performed in two conditions of inflammatory challenge, thermal injury and RA. sGPVI levels were elevated in RA, whereas there was no significant increase in sGPVI after initial thermal injury compared to healthy controls. In contrast, peak sGPVI levels were greater in burns patients with sepsis compared to non-septic patients ($P < 0.01$). CLEC-2 levels in RA patients were not increased compared to healthy controls, possibly because CLEC-2 is retained on microvesicles. There was an increase in podoplanin expression on microvesicles in patients with RA.

Conclusion: sGPVI is a biomarker for platelet activation in RA and during sepsis progression in patients with thermal injury. Further work is required to establish the potential of CLEC-2 and podoplanin as biomarkers of inflammation.

Disclosure of Interest: None declared.

PO394-TUE

Novel whole blood model of thrombocytopenia with preserved platelet function

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Background: *In vitro* models of thrombocytopenia are useful research tools. Previous methods have shortcomings altering properties of platelets and other blood components.

Aims: To develop a whole blood-based method to induce thrombocytopenia with minimal manipulation of blood cells and to describe platelet function of healthy platelets in induced thrombocytopenia.

Methods: The Regional Research Ethics Committee approved the study. Hirudin anticoagulated blood was collected from 20 healthy volunteers after informed consent. One part of the blood was gently centrifuged at 130 g for 15 minutes. The platelet-rich plasma was replaced with phosphate-buffered saline to establish thrombocytopenia. Various levels of thrombocytopenia were achieved by combining predefined volumes of baseline whole blood and thrombocytopenic blood. Platelet counts were measured by flow cytometry. Platelet function was analysed by impedance aggregometry and by flow cytometry

using collagen, adenosine diphosphate, thrombin receptor agonist peptide-6 and ristocetin as agonists for both methods.

Results: Median baseline platelet count was $227 \times 10^9 \text{ L}^{-1}$. The *in vitro* model yielded median platelet counts as low as $51 \times 10^9 \text{ L}^{-1}$. We observed minor, yet significant, changes in platelets from baseline to thrombocytopenia (before vs. after): mean platelet volume (10.2 vs. 10.8 fL) and immature platelet fraction (4.1 vs. 6.6%) (P -values < 0.005). In the thrombocytopenic samples, we observed strong positive linear correlations between platelet count and platelet aggregation in all agonists (all P values < 0.001). Platelet function by flow cytometry showed small variations for all agonists before and after manipulation, verifying that platelet function did not change after manipulation.

Conclusion: We established and validated a new whole blood-based model of thrombocytopenia with preserved platelet function. This new model may serve as a useful tool to explore platelet function in patients with thrombocytopenia.

Disclosure of Interest: None declared.

PO395-TUE

Local anaesthetics inhibit activation of Protein Disulfide Isomerase (PDI)

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Background: Regional anaesthesia has been shown to be associated with reduced incidence of post-operative thromboembolism. Protein disulfide isomerase (PDI), an oxido-reductase and chaperone, participates in integrin activation and fibrin formation. It is crucially involved in thrombosis and inflammation. Inhibition of PDI using neutralizing antibodies blocked thrombus formation in mouse models.

Aims: The aim of the research was to study the effect of local anaesthetics on PDI activity.

Methods: The effect of ester-type and amide-type local anaesthetics ($20\text{--}200 \mu\text{g mL}^{-1}$) on platelet activation by the neutrophil extracellular trap component HOCl modified albumin was measured by flow cytometry. Extracellular disulfide reductase activity on platelet surface was determined according to the fluorescent assay from Raturi and Mutus. PDI reductase activity in solution using purified PDI was performed using the PDI substrate di-eosin-oxidized-glutathione. Fluorescence was measured in a Fluoroscanner-Ascent-fluorimeter.

Results: The ester-type local anaesthetic procaine and the amide-type local anaesthetics lidocaine, prilocaine, mepivacaine, bupivacaine and ropivacaine ($20\text{--}200 \mu\text{g mL}^{-1}$) inhibited in dose dependent manner platelet activation by HOCl-modified albumin. In addition extracellular PDI reductase activity on platelet surface and purified PDI in solution were inhibited by all tested local anaesthetics dose dependently.

Conclusion: Inhibition of thiol disulfide isomerase is likely to contribute to the beneficial effect of local anaesthetics in the protection against thromboembolism.

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PO396-TUE

Prospective analysis of platelet activation markers to predict severe infection and mortality in intensive care units

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Background: Sepsis is often diagnosed too late due to non-specific nature of its signs and symptoms. Early diagnosis using specific blood biomarkers could better guide antibiotic therapy and importantly improve patient outcome.

Aims: We evaluated platelet activation markers as potential predictive markers of sepsis and of mortality among 3 commonly encountered populations of patients admitted to intensive care units (ICUs).

Methods: 99 non-infected patients were prospectively screened at day 1–2 (T1) and day 3–4 (T2) of admission after elective cardiac surgery, trauma, or acute neurologic dysfunction. A third sample was drawn when infection was diagnosed (Tx). We evaluated platelet activation by measuring the expression of P-selectin (CD62P) and fibrinogen binding on cell surface in flow cytometric assays. Clinical scores (SOFA, APACHE II) were recorded at admission.

Results: Patients who developed sepsis presented with significantly higher platelet fibrinogen binding at T1 compared with patients who did not get infected ($P = 0.0014$, ROC AUC = 0.79 [95% CI: 0.68–0.89]). Elevated basal CD62P expression level was associated with increased 90-day mortality ($P = 0.042$, ROC AUC = 0.78 [95% CI: 0.64–0.88]). Kaplan-Meier survival curves illustrated that mortality was significantly different after stratification based on T1 basal CD62P level (cut-off MFI >31.56, hazard ratio 13.6, $P = 8.23 \times 10^{-6}$). Multivariate logistic regression analysis using clinical scores indicated that addition of CD62P levels or of bound fibrinogen levels significantly improved prediction of mortality (odds ratio 1.078, $P = 0.003$) and sepsis (odds ratio 1.033, $P = 0.0012$), respectively.

Conclusion: Predisposition to severe infection in critically ill medico-surgical adults can be identified on day 1–2 of admission based on circulating basally activated platelets. Levels of activated platelets may add incremental prognostic information to clinical scoring.

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PO397-TUE

Staphylococcus aureus lipoproteins inhibit platelet activation

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Background: *Staphylococcus aureus* is an opportunistic pathogen known for its ability to interact with platelets and is a major cause of bacterial endocarditis. *S. aureus* surface molecules are known to interact with platelet surface molecules and modulate platelet function. *S. aureus* lipoproteins (LP) are one of the major bacterial surface molecules that are released into the extracellular milieu and are recognised by host immune cells.

Aims: To determine the role of *S. aureus* LP in *S. aureus* – platelet interaction.

Methods: *S. aureus* wild type and *S. aureus* *lgt* mutant (lacks lipidation of LP) strains were used for aggregometry and binding assay. *S. aureus* LP were extracted by TX-114 phase partitioning method. Flow cytometry using FITC tagged *S. aureus* LP extracted from *S. aureus* LTA and *lgt* strains was done to check its binding to platelets. Platelets were pre-incubated with *S. aureus* LP and spreading on fibrinogen, aggregation response, fibrinogen binding and P-selectin exposure on agonist stimulation was checked. Biotinylated *S. aureus* LP were incubated with platelets and detected by blotting on co-immuno-precipitation with CD36. Platelet aggregation and spreading on fibrinogen was performed by pre-incubating CD36 neutralized platelets with *S. aureus* LP.

Results: Platelet aggregation and binding assays showed *S. aureus* LP to be involved in binding to platelets, which was confirmed by flow cytometry showing binding of FITC labelled *S. aureus* LP to platelets. Pre-incubation of platelets with *S. aureus* LP inhibited platelet spreading and agonist induced platelet aggregation, P-selectin exposure and fibrinogen binding. Co-immuno-precipitation showed CD36 as a platelet surface protein binding to *S. aureus* LP and inhibitory effect of LP on platelet spreading and aggregation was abrogated on treating platelets with anti-CD36 monoclonal antibody.

Conclusion: Platelet activation is inhibited by *S. aureus* LP and this inhibition involves platelet CD36.

Disclosure of Interest: None declared.

PO398-TUE

Comparative impacts of antiretroviral HIV therapies on platelet function

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Background: Highly active antiretroviral therapy (HAART) has considerably improved the life expectancy of HIV-infected individuals. However, therapeutics such as the nucleoside reverse transcriptase inhibitor (NRTI) abacavir sulphate, are reportedly associated with increased risk of thrombotic events such as myocardial infarction (MI).

Aims: The aim of this study was to assess the impact of two NRTIs, abacavir sulphate and tenofovir on platelet aggregation *in vitro* and *in vivo*.

Methods: Isolated human platelets from healthy volunteers were incubated at various time points with either abacavir sulphate ($3 \mu\text{g ml}^{-1}$) or tenofovir ($3 \mu\text{g ml}^{-1}$). Platelets were stimulated with collagen ($0.1\text{--}2.5 \mu\text{g ml}^{-1}$) or thrombin ($0.01\text{--}0.1 \text{ U ml}^{-1}$) and aggregation was measured using optical platelet aggregometry.

Platelet aggregation in response to a submaximal dose of collagen ($50 \mu\text{g kg}^{-1}$) was measured in mice following an acute (30 minutes) dose of abacavir sulphate or tenofovir (estimated plasma concentration of $30 \mu\text{g ml}^{-1}$).

Results: Tenofovir significantly inhibited platelet aggregation induced by an EC₅₀ concentration of thrombin (0.03 U ml^{-1}) and collagen ($0.6 \mu\text{g ml}^{-1}$) compared to the vehicle control *in vitro*, in contrast no effect was detected following incubation with abacavir sulphate.

Abacavir sulphate significantly enhanced collagen induced platelet aggregation compared to the vehicle control *in vivo*. No effect was observed following treatment with tenofovir.

Conclusion: Abacavir sulphate enhanced platelet aggregation *in vivo* which suggests that NRTIs can influence platelet behaviour and therefore the increased risk of MI which has been associated with abacavir sulphate may be platelet driven. In contrast, tenofovir negatively affected agonist-induced platelet aggregation *in vitro*, suggesting that

this NRTI may protect against platelet-driven cardiovascular events. This data demonstrates that further work is warranted in this field.

Disclosure of Interest: None declared.

PO399-TUE

Analyzing *in vitro* platelet thrombus formation of patients with essential thrombocythemia under blood flow conditions

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Background: Thrombosis is a leading cause of death and disability worldwide. Essential thrombocythemia (ET) is a myeloproliferative neoplasm characterized by an increased platelet count, megakaryocytic hyperplasia, and thrombotic tendency. Although elevated platelet count may increase the risk of thrombosis, extreme thrombocytosis may also be associated with bleeding due to an acquired deficiency of von Willebrand factor (VWF). Several laboratory tests for platelet function have recently become clinically available. However, it is still unclear whether these tests actually reflect *in vivo* risks of thrombosis and bleeding due in part to the absence of blood flow and the addition of a single exogenous agonist at a supra-physiological concentration. Recently, we developed a novel microchip-based total thrombus-formation analysis system (T-TAS) that can quantitatively assess the process of obstructive thrombus formation under blood flow.

Aims: In this study we analyzed platelet functions of patients with ET using T-TAS.

Methods: Blood samples were collected from patients with ET, and whole blood anticoagulated with hirudin was perfused over a microchip coated with collagen. The processes of platelet thrombus formation inside the microchip were analyzed by T-TAS in which a video microscope and a flow pressure sensor were equipped.

Results: The growth of platelet thrombus and the occlusion of collagen-coated capillaries were accelerated in patients with ET compared with healthy volunteers. However, in a case with platelet count of 2 million μL^{-1} , the growth of thrombus at fast-flowing sites were inhibited possibly as a result of acquired deficiency of VWF, and the microchip capillary did not occlude in this case. Therapy with aspirin decreased the stability of platelet thrombus.

Conclusion: T-TAS may be useful in evaluating multifunction of platelets, including adhesion, activation, and aggregation.

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PO400-TUE

Generation of procoagulant coat platelets in stored platelet-concentrate units derived from buffy-coat preparations

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Background: COAT platelets, generated by dual agonist stimulation with collagen and thrombin, efficiently sustain thrombin generation. Higher amounts of COAT platelets are associated with stroke, while a decreased ability to generate COAT platelets is associated with bleeding diathesis. Platelet concentrates (PC) can be obtained from the buffy-coat of whole blood donations or by aphaeresis.

Aims: This work aims to study platelet functions, particularly the ability to generate COAT platelets in PC from donors prepared with the buffy-coat method (BC-PC) and to investigate whether the level of COAT platelets in BC-PC is modified either by pathogen inactivation Intercept treatment or by prolonged storage.

Methods: BC-PC platelets were analyzed by flow cytometry before pathogen inactivation and during storage. Two BC-PC from 5 donors each were pooled and split in two bags, one of them was pathogen inactivated by Intercept process and the other one was left untreated. We measured the efficiency of BC-PC to generate COAT platelets following stimulation with thrombin and convulxin in treated and untreated BC-PC. Additional parameters were platelets surface receptors, expression of P-selectin and PAC1-binding at baseline and after increasing doses of agonists and content/secretion of dense granules.

Results: Among the parameters measured, a distinguishing feature that appears is the decrease of COAT platelets in BC-PC platelets compared to platelets from platelet-rich plasma. While in untreated BC-PC the decrease of COAT platelets is constant over time, in Intercept-treated BC-PC the COAT platelet loss appears to progress during storage and is complete at day 10.

Conclusion: BC-PC platelets preparation seems to decrease the ability to generate procoagulant COAT platelets. Pathogen inactivation by Intercept treatment appears to further impair the formation of COAT platelets. The clinical relevance of this observation is unknown. Current work aims at confirming and identifying the mechanisms underlying this phenomenon.

Disclosure of Interest: None declared.

PO401-TUE

Opiates may attenuate platelet activation

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Background: Morphine and other opiates are widely used for pain treatment in a variety of conditions. Opiates also affect respiratory function, sedation, diuresis and the functioning of the gastrointestinal tract. The μ , δ and κ opioid receptors are involved in mediating the effects of opiates through pertussis-sensitive G-proteins. However, the effect of opiates on platelet aggregation is poorly studied. Since most patients receiving pain relief also require a functional hemostatic system, it is important to understand their impact on coagulation and characterise their individual effects, dose response and potency.

Aims: To characterize dose-response effects of five commonly used opiates; tramadol, fentanyl, oxycodone, morphine and ketobemidone on platelet aggregation, and activation.

Methods: The expression of the activation markers P-selectin and fibrinogen was analyzed by flow cytometry. Platelet aggregometry was assessed by impedance technology. Western blotting was used to measure the level of AKT phosphorylation at Ser 473 residue.

Results: The higher dose of tramadol significantly reduced both P-selectin and fibrinogen expression irrespective of stimuli. Oxycodone significantly inhibited CRP stimulated expression of P-selectin and fibrinogen and Trap-6 stimulated expression of fibrinogen. Ketobemidone inhibited up-regulatory effects of all stimuli studied with the exception of CRP induced P-selectin expression. Morphine and fentanyl had no effect in the two doses examined. The higher dose of tramadol, ketobemidone and oxycodone all resulted in a significant reduction in the aggregation response by platelets. Western blot analysis showed that agonist treated samples in presence of opiates had a decrease in Ser 473 phosphorylation compared to samples treated with agonist alone.

Conclusion: Tramadol, ketobemidone and oxycodone significantly inhibited platelet aggregation and activation as judged by expression of P-selectin and fibrinogen.

Disclosure of Interest: None declared.

PO402-TUE

***In vivo* and protease-activated receptor 1-mediated platelet activation in patients presenting for cardiac catheterization**

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Background: Alternative pathways of platelet activation, which are not targeted by current antithrombotic therapy, may be crucial for the development of ischemic events in patients with cardiovascular disease.

Aims: To investigate whether *in vivo* and protease-activated receptor-1 mediated platelet activation and monocyte-platelet aggregate (MPA) formation can serve as independent risk markers for future adverse outcomes in aspirin-treated patients presenting for cardiac catheterization.

Methods: *In vivo* and thrombin receptor-activating peptide (TRAP)-stimulated platelet surface P-selectin expression, platelet surface activated glycoprotein IIb/IIIa (GPIIb/IIIa) and MPA formation were determined in 682 consecutive adult patients undergoing cardiac catheterization and in 47 healthy controls. Two-year follow-up data were obtained from 562 patients (82.4%).

Results: *In vivo* platelet surface P-selectin, platelet surface GPIIb/IIIa and MPA formation were significantly higher in patients than in healthy controls (all $P \leq 0.02$). Patients with an acute coronary syndrome (ACS) had significantly higher levels of *in vivo* MPA formation than patients without ACS ($P = 0.01$). In the overall study population ($n = 562$, 100%) the platelet surface expression of P-selectin and activated GPIIb/IIIa, and the formation of MPA *in vivo* and in response to TRAP were similar in patients without and with subsequent ischemic events (all $P > 0.05$). Comparable results were obtained when only patients with angiographically-proven coronary artery disease ($n = 459$, 81.7%) or ACS ($n = 125$, 22.3%) were analyzed. Receiver operating characteristic curve analyses did not reveal cut-off values for *in vivo* and TRAP-stimulated P-selectin, activated GPIIb/IIIa, and MPA formation for the prediction of future ischemic events.

Conclusion: *In vivo* and TRAP-stimulated platelet activation and MPA formation did not predict adverse ischemic outcomes in aspirin-treated patients presenting for cardiac catheterization.

Disclosure of Interest: T. Gremmel: None declared, A. Michelson Grant/Research Support from: Siemens, Lilly/Daiichi Sankyo, Pfizer, Bristol-Myers Squibb, GL Synthesis, Consultant for: Lilly, A. Frelinger Grant/Research Support from: Siemens, Lilly/Daiichi Sankyo, Pfizer, Bristol-Myers Squibb, GL Synthesis.

PO403-TUE

Platelet Factor-3 (PF-3) availability and platelet count of pregnant women in irrua, edo state

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Background: Platelets are small fragments of cytoplasm derive from megakaryocyte which functions in the blood clotting process as they release their clotting factor (known as platelet factor-3), whose availability serve as an invitro platelet function test as it gives a direct measurement of the platelet release reaction.

Aims: This study aimed at assessing the influence of normal uncomplicated pregnancy on the platelet count and platelet factor-3 availability and in determining the extent of relationship between gestation period (trimester) and age on the platelet count.

Methods: The platelet count and platelet factor-3 (PF-3) availability of 70 pregnant women and 50 non-pregnant women (control) were carried out using standard methods.

Results: The mean platelet count among the pregnant women (344 ± 95.83) was statistically higher than that of the non-pregnant controls (292 ± 137.02) ($P < 0.05$). the PF-3 availability value for the control was 63 ± 27.67 as against the reference value of 29–54 s that has been previously quoted. The PF-3 availability was found to be reduced in normal pregnancy. There was no statistical significance of the effect of age and trimester on the platelet count and PF-3 availability ($P > 0.05$).

Conclusion: It was therefore concluded that pregnancy is associated with a significant increase in platelet count and that functional clotting activity of platelet factor-3 may not be limited to the number of platelets.

Disclosure of Interest: None declared.

PO404-TUE

Flow cytometry based evaluation of platelet-neutrophil complex formation in human and murine whole blood: a detailed analysis of ex vivo sample preparation, cytometer settings and molecular basics

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Background: The formation of platelet-neutrophil complexes (PNCs) is found in states of inflammation and hemostasis and thus is regarded as a keyplayer in a wide range of diseases. Platelet P-selectin and its ligand PSGL-1 are crucial for complex formation. Although multiple publications have reported PNC counts, we noticed that different methods were used to analyze PNC formation and results were presented variably.

Aims: To establish a detailed, reproducible and preferably simple protocol for PNC measurement by flow cytometry in murine and human whole blood, we compared different sample preparation and cytometer settings.

Methods: Whole blood from healthy donors or WT mice was analyzed by flow cytometry. PNC levels in diluted blood (1:2 vs. 1:6) were quantified after treatment with vehicle, 20 μ M ADP or 100 nM PMA using a three colour staining with CD45 for leukocytes, Ly6G(Gr1) or CD15 for neutrophils and CD41 for platelets. High versus low cytometer speed was compared. Effect of P-selectin blocking by antibody was examined. Platelet activation was determined by antibody against activated GPIIb/IIIa.

Results: Baseline PNC levels were $16.1 \pm 5\%$ ($P < 0.005$) in murine and $7.2 \pm 3\%$ ($P < 0.005$) in human samples (dilution 1:6, low reading speed). Low flow rate and dilution 1:2 increased PNCs about 7% in murine blood but did not affect human PNC levels. High reading speed lead to artificially high PNC counts. Treatment with ADP or PMA resulted in $30.7 \pm 5\%$ and $99 \pm 0.3\%$ in mice ($P < 0.05$), $25 \pm 10\%$ and $60 \pm 15\%$ in humans ($P < 0.05$), respectively (1:6, low flow rate). P-selectin blocking abolished PNC formation after ADP in both species and reduced PMA induced complex formation in human blood. However it had no effect on PMA stimulated murine samples. Evaluation of platelet activation was similar in both mice and humans. **Conclusion:** In murine and human samples best results could be reached by diluting whole blood 1:6 and using low flow rate. P-selectin blocking could not prevent PNC formation in murine PMA treated blood.

Disclosure of Interest: None declared.

PO405-TUE

T2MR platelet analysis correlates with LTA and reveals unique details of ADP-mediated platelet activation in whole blood

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Background: There is a need for simple, rapid platelet activity diagnostics with similar or improved performance relative to the established reference method, light transmission aggregometry (LTA). for this purpose we present new data characterizing the T2 magnetic resonance (T2MR) hemostasis methodology that is being developed for the assessment of platelet-mediated clot contraction in whole blood.¹

Aims: Our aim was to assess the ability of T2MR to discriminate platelet activity between donors with normal and impaired platelet function, defined by LTA.

Methods: Citrated whole blood was collected from 20 healthy donors not taking anti-platelet medications. The reagent formulation contained reptilase to activate fibrin and heparin to inhibit thrombin. Platelets were activated with 5–20 μ M ADP, 0.5–1.5 mM arachidonic acid (AA), 1–10 μ M TRAP, or 5–20 μ M epinephrine.¹ To assess specificity, agonists were combined with their antagonists.

Results: Correlation between T2MR and LTA demonstrated a positive percent agreement of 98% and a negative percent agreement of 97%. Recovery of platelet function after 325 mg aspirin was comparable when measured by both methods. T2MR and LTA both indicated a lack of platelet activity from a patient with Glanzmann's thrombasthenia. Notably, ADP responses from T2MR results were only partially inhibited with MeSAMP alone, in contrast to LTA, but completely inhibited by addition of MRS2279 with MeSAMP. This suggests that ADP-mediated platelet activation is more dependent on P2Y1 signaling in whole blood when compared to platelet-rich plasma used for LTA.

Conclusion: The T2MR platform requires 40 μ L of whole blood, is simple and provides results within 20 minutes that are highly correlated with LTA. It also provides insight into platelet function in whole blood not observed with LTA, demonstrating the potential importance

of platelet function data that is integrated into clot contraction studies in whole blood that cannot be assessed by LTA.

Reference: 1. Skewis *Clin Chem* 2014 60:1174.

Disclosure of Interest: None declared.

PO406-TUE

New approach for evaluation of platelet function in thrombocytopenia using impedance aggregometry

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Background: Flow cytometry has been introduced for evaluation of platelet function independent of platelet count. Whole blood aggregometry is a quicker and more widely available functional assay, but has until now, not been considered feasible at low platelet counts.

Aims: To evaluate platelet function using impedance aggregometry adjusted for platelet count in thrombocytopenia and compare the findings to platelet function assessed by flow cytometry.

Methods: The Regional Research Ethics Committee approved the study. Following informed consent, hirudin anticoagulated blood was collected from 20 healthy individuals, 17 haematological cancer patients and 20 patients with primary immune thrombocytopenia (ITP). Platelet function was analysed by impedance aggregometry and by flow cytometry. for both analyses collagen, adenosine diphosphate and thrombin receptor agonist peptide-6 were used as agonists. Platelet aggregation was adjusted for platelet count relative to thrombocytopenia induced in healthy whole blood employing a novel validated method.

Results: Platelet counts were obtained in similar ranges ($13\text{--}129 \times 10^9 \text{ L}^{-1}$) for each group ($P = 0.75$). Aggregation adjusted for platelet count was significantly reduced in cancer in all agonists (median: 23–33% of healthy, P -values < 0.02). In contrast, the platelet aggregation in ITP was increased (223–308% of healthy, P -values < 0.01). ITP displayed increased expression of bound fibrinogen and CD63 following activation, compared to particularly cancer patients, but also healthy platelets. The expression of platelet surface markers following activation with all agonists showed moderate correlation to platelet aggregation adjusted for platelet count in thrombocytopenia (Spearman's rho 0.28–0.63, P -values ≤ 0.04).

Conclusion: The new approach made it possible to differentiate platelet function of healthy individuals and two different patient groups. This study demonstrated the feasibility to perform platelet function analyses in thrombocytopenia using impedance aggregometry.

Disclosure of Interest: None declared.

PO407-TUE

Development and validation of a flow cytometry assay for assessing cyclooxygenase-1 and 2 in platelets

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Background: A reduced effect of aspirin has been reported in patients with a high immature platelet fraction (IPF). Cyclooxygenase (COX)-1 is present in all platelets and is inhibited by aspirin. Immature platelets also contain COX-2, which is not affected by the low doses of aspirin used in the treatment of cardiovascular disease.

Aims: To develop and validate an assay to assess COX-1 and COX-2 in platelets, and to investigate the association with IPF.

Methods: Sodium citrated platelet rich plasma was fixed and permeabilised and antibodies for CD41, CD45, COX-1 and COX-2 were added. The solution was incubated for 30 min, washed and diluted prior to flow cytometric analysis. Isotype controls were used as negative control, CD41 was used to identify platelets and CD45 for eliminating leucocytes, and leucocyte-platelet aggregates. Thirty healthy volunteers and 15 patients with primarily immune thrombocytopenia patients (ITP) will be included. Informed consent is obtained from all participants, and the study has been approved by The Central Denmark Region Committees on Health Research Ethics and will be conducted in accordance with the Helsinki II Declaration.

Results: Preliminary results of two healthy volunteers with IPF of 3–5% showed detectable levels of COX-1 [6–9 medium fluorescence intensity (MFI)] in all platelets, but only 3–10% of platelets contained COX-2. Preliminary results of three ITP patients with IPF ranging from 8 to 28% showed increased levels of COX-1 in all platelets (9–26 MFI) and fraction of platelets containing COX-2 (9–28%). Reference intervals for COX-1 and COX-2 will be established on healthy volunteers and compared with ITP patients.

Conclusion: We succeeded in developing and validating an assay for assessment of COX-1 and COX-2 in platelets. Investigating the association between COX-1, COX-2 and IPF is of high interest as it may contribute to the explanation of why a reduced effect of aspirin is found in patients with a high IPF.

Disclosure of Interest: None declared.

PO408-TUE

Platelet aggregation and biological response modifiers release by combinations of *Streptococcus sanguinis* and *Porphyromonas gingivalis*

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Background: During periodontal disease, bacteria and their by-products elicit inflammation of the periodontium, leading to an increased incidence of bacteraemias. Evidence suggests these bacteraemias are positive risk factors for cardiovascular disease. Certain strains of *Streptococcus sanguinis* (*S. sanguinis*) and *Porphyromonas gingivalis* (*P. gingivalis*) can activate platelets in a donor-dependant manner and thus are potential contributing factors in thromboembolic disease. While these pathogens have been studied individually, the effects of combinations of pathogens, as would occur during a bacteraemia, have not been reported.

Aims: The aim of the current study was to determine whether there was synergistic action of *S. sanguinis* and *P. gingivalis* on platelet activation.

Methods: Aggregation of human platelets was determined by increased light transmission of platelet rich plasma, and the release of the biological response modifiers (BRMs) sCD40L, sCD62p and RANTES was quantified by ELISA.

Results: Aggregation was observed in platelets in response to three strains of *S. sanguinis* (2017–78, SK112, SK108a) and three strains of *P. gingivalis* (W50, W83, 6.26). Sub-threshold levels of 2017–78 significantly decreased the lag-time to full aggregation, but had no effect on the release of BRMs, when combined with sub-threshold levels of each *P. gingivalis* strain. These data demonstrate that platelet aggregation, but not the release of BRMs, can occur readily in response to a heterogenic bacterial challenge.

Conclusion: Platelet activation in response to a total pathogenic burden may contribute to the association of periodontitis with an acute thrombotic event, whereas a challenge with multiple organisms does not enhance platelet-mediated inflammatory responses. These data are

also consistent with distinct regulatory pathways contributing to the thrombotic (i.e., aggregation) and inflammatory (i.e., secretion of BRMs) processes.

Disclosure of Interest: None declared.

PO409-TUE

Increase in ionic strength inhibits platelet aggregation irrespective of solute type in vitro

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Background: Haemodilution with hypertonic solutions impair whole blood coagulation but not plasma clotting tests. The effects of crystalloid diluents composition on platelet aggregation remain largely unexplored.

Aims: To investigate effects of crystalloid diluents solute composition and concentration on platelet aggregation.

Methods: PRP and PPP was obtained from 10 healthy donors, divided into aliquots and haemodiluted with selected diluents at increasing concentrations. Platelet aggregation was stimulated with ADP and AA and measured in Helena Aggram aggregometer.

Results: Platelet aggregation decreased dose dependently according to ionic strength irrespective of solute type.

%Max aggregation per diluents concentration in AA Agonist

Diluents/conc	300 mOsm	600 mOsm	1000 mOsm	1500 mOsm	2500 mOsm
undiluted	76.3	76.3	76.3	76.3	
Na gluconate	58.1	–	24.5	12.2	
NaCl	76.8	55.5	38.9	16.3	
Choline chloride	61.3	–	33.7	13.8	
Mannitol	65.5	–	49.6	–	
Dextrose	57.1	–	–	–	10.5

Conclusion: Platelet aggregation is inhibited by increase in ionic strength and osmolality suggesting osmosensory role.

Disclosure of Interest: None declared.

PO410-TUE

A new approach to measuring platelet aggregation and platelet-leucocyte conjugate formation in a small volume of fixed whole blood

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Background: Platelet function testing is of value in determining the relative impact of different platelet agonists and inhibitors on platelet function. Investigations can be limited by the volume of blood that is available.

Aims: To assess platelet aggregation (PA) and platelet-leukocyte conjugate (PLC) formation in a small blood volume using a 96-well plate format.

Methods: Platelet function was assessed in whole blood from healthy volunteers using 96-well plates coated with: arachidonic acid (AA, 0.03–1 mM), ADP (0.3–30 µM), collagen (0.1–10 µg mL⁻¹) and TRAP (0.1–10 µM). To check the suitability of this assay to detect platelet inhibition the effects of aspirin, the P2Y₁₂ inhibitor cangrelor, the GPIIb/IIIa blocker MK-852 and the PSGL-1 blocker KPL-1 were investigated. Whole blood (46 µl) was added to each well and the plate was shaken for 5 min at 1000 rpm at 37 °C; a fixative solution AGG-

Fix (Platelet Solutions Ltd, Nottingham, UK) was applied to stop platelet stimulation and stabilise samples. Both PA, as a fall in the number of single platelets, and PLC formation were measured in the same fixed sample by flow cytometry without red cell lysis and within 3 days after fixation.

Results: Full dose-response curves to 4 agonists were generated using 1.2 ml of whole blood. Aspirin inhibited AA-induced PA, cangrelor induced dose-dependent inhibition of ADP-induced PA and both agents inhibited PLC formation. Collagen- and TRAP-induced PA was also impaired to a different extent by *in vitro* addition of either antiplatelet agent. PLC formation was readily measured in the same samples as PA and was abolished by the addition of KPL-1, which had no effect on PA. In the presence of MK-852 PA was not completely inhibited while PLC formation was dramatically increased and the latter was only partially inhibited by further addition of KPL-1.

Conclusion: The assessment of PA and PLC formation in one fixed whole blood samples offers a reliable method for obtaining two measures of platelet function using a very small volume of blood.

Disclosure of Interest: M. Algahtani: None declared, N. Dovlatova Employee of: Platelet Solutions Ltd., J. May Shareholder of: Platelet Solutions Ltd., S. Heptinstall Shareholder of: Platelet Solutions Ltd., S. Fox Shareholder of: Platelet Solutions Ltd.

PO411-TUE

The automation of routine light transmission platelet aggregation on sysmex CS-2000i

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Background: Light Transmission aggregometry requires specific equipment and qualified operators; the volume of sample required can also be restricting especially for pediatric analysis. In order to improve the implementation of these techniques in the laboratory of hemostasis, we assessed the ability of a high throughput coagulation analyser CS-2000i (Sysmex Corporation, Japan) with specific software to perform platelet aggregation.

Aims: Assessment of the ability of a CS-2000i with specific software to perform platelet aggregation in various clinical samples.

Methods: 50 individuals were studied in total. Samples were from healthy subjects, patients with congenital platelet disorders or treated with anti-platelet drugs. Platelet aggregation were performed on CS-2000i equipped with dedicated software and PAP-8E (Biodata, USA) as the reference instrument. The agonists for the CS-2000i: ADP 2 μ M, Collagen 2 μ g mL⁻¹, Epinephrine 5 μ M, Arachidonic Acid 1 mM and Ristocetin 1.5 mg mL⁻¹ from HYPHEN BioMed (France) were used. for the PAP-8E, the agonists: ADP (Sigma, USA) 2 μ M, Collagen Horm (Takeda, Austria) 2 μ g mL⁻¹, Arachidonic acid (Bio Data, USA) 1 mM, and Ristocetin (Stago, France) 1.5 mg mL⁻¹ were used. PRP were used on both devices at the same time which standardized $\sim 250 \times 10^9$ L⁻¹ with autologous plasma. Stirrer speed were 800 rpm for the CS-2000i and 1200 rpm for the PAP-8E.

Results: CS-2000i using agonists from HYPHEN BioMed were comparable performances with those obtained by PAP-8E instrument using various agonists. The reason of dispersions especially with ADP 2 μ M are explained by some discrepant plasmas, which could exhibit variable aggregation patterns for low responsive samples in their specific profiles.

Conclusion: Platelet aggregation on CS-2000i allow a fast and standardized method for light transmission aggregometry. Another advantage of this automation is the lower sample volume required which can be useful for pediatric patients. This automation facilitates the realization of these specific assays in many of laboratories.

Disclosure of Interest: K. Kobayashi Employee of: HYPHEN BioMed, P. Gueret: None declared, M. Sansot: None declared, F. Nedelec Gac: None declared, J. Amiral Consultant for: HYPHEN BioMed and Sysmex.

PO412-TUE

Effects of pH and concentration of sodium citrate anticoagulant on platelet aggregation measured by light transmission aggregometry induced by adenosine diphosphate

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Background: The SSC-ISTH guidelines for standardisation of light transmission aggregometry (LTA) for the study of platelet aggregation (PA) were largely based on the consensus of experts due to lack of studies directly comparing different procedures.

Aims: We experimentally tested the cogency of some SSC-ISTH recommendations: 1) whether buffering the anticoagulant keeps pH of PRP samples stable and affects PA; 2) whether the 2 recommended concentrations of sodium citrate (109 and 129 mM) are equivalent in terms of PA response; 3) whether time dependence of PA induced by ADP is equivalent in buffered and non-buffered citrate.

Methods: Blood from 16 healthy volunteers was collected into buffered and non-buffered citrate 109 and 129 mM. PA was measured by LTA in PRP stimulated by ADP (2 μ M) 45 min (immediately after preparation), 1, 2, 3 and 4 h after blood sampling. Plasma pH was measured at the same time points.

Results: pH in PRP increased with time for all anticoagulants; in buffered citrate, pH was significantly lower than in non-buffered citrate at all times. PA induced by ADP was lower in buffered citrate at 45 min, but equivalent at all other times. PA was higher in sodium citrate 109 mM than in 129 mM at all times. PA was time-dependent for all citrate preparations, with lower responses at 45 min, stable maximum responses at 1 and 2 h, and subsequent decrease at 3 and 4 h.

Conclusion: Buffering sodium citrate anticoagulant lowers the pH of whole blood and PRP samples to a more physiological value, but does not keep pH stable over time. Buffering anticoagulant has no effect on PA after PRP is allowed to rest for 15 min. PA is higher in samples collected in sodium citrate 109 mM compared to 129 mM. PA induced by ADP is highly time-dependent in all citrate anticoagulants tested and diminishes significantly 3 h after blood sampling; therefore, studies of PA should ideally be completed within 2 h of blood collection.

Disclosure of Interest: None declared.

PO413-TUE

Anti-protamine/heparin antibodies induced platelet activation in the presence of NPH-insulin in heparin dependent manner

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Background: Immunization against protamine/heparin (PRT/H) complexes is common in patients undergoing cardiac surgery. PRT is used in Neutral Protamine Hagedorn (NPH) insulin, a commonly used therapeutic agent for diabetes mellitus.

Aims: To analyze the interaction between NPH insulin and anti-PRT/H antibodies and its impact on platelet (PLT) activation.

Methods: Flow cytometry (FC) was used to study the binding of NPH-Insulin to PLTs and to investigate the ability of anti-PRT/heparin antibodies to activate PLTs in the presence of NPH-insulin and Heparin.

Results: NPH insulin, but not native insulin, binds to PLTs. The binding was enhanced by heparin (NPH insulin: median mean fluorescence

intensity (MFI) 16, range 15–18 vs. NPH insulin and unfractionated heparin (UFH): median MFI 24, range 17–29). Anti-PRT/H antibodies from immunized patients bound to platelets in the presence of NPH insulin, but not in the presence of native insulin. This binding was also enhanced by the addition of UFH (NPH insulin: median MFI 33, range 18–37 vs. NPH insulin and UFH: median MFI 65, range 15–78). The ability of anti-PRT/H antibodies ($n = 6$) to activate NPH insulin-coated platelets was investigated by measuring the P-selectin (CD62p) expression using FC. Anti-PRT/H antibodies activated PLTs in the presence of NPH insulin in a heparin-dependent way (CD62p expression; NPH insulin (mean $\pm$ standard deviation [SD]): MFI 16 ± 3 vs. NPH insulin and UFH: MFI 427 ± 323).

Conclusion: Our data demonstrate the ability of NPH insulin to bind platelets in a heparin-dependent way. Anti-PRT/H antibodies recognize bound PRT and induce platelet activation. These *in vitro* findings justify further investigations to assess the clinical impact of the interaction between anti-PRT/H antibodies and NPH insulin.

Disclosure of Interest: None declared.

PO414-TUE

Characterising cell-type interactions following platelet activation in a novel low volume whole blood assay

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Background: Platelet-platelet interactions are central to the processes of haemostasis, while platelet-neutrophil and platelet-monocyte interactions have been linked to a range of cardiovascular disease states. Study of these interactions is technically challenging, particularly in genetically modified mice and in small volume clinical samples such as from children.

Aims: Here we have addressed this problem by coupling whole blood stimulation, using a low volume 96-well plate approach, with flow cytometric imaging.

Methods: In brief, blood was collected by venepuncture of the vena cava in anaesthetised C57bl6 mice or the antecubital vein of healthy volunteers into hirudin ($10 \mu\text{g mL}^{-1}$ final). $35 \mu\text{L}$ aliquots of blood were then placed into the individual wells of half-area 96-well plates, with or without agonist (collagen, $1\text{--}30 \mu\text{g mL}^{-1}$; PAR-activating peptides, $1\text{--}100 \mu\text{M}$; U46619, $0.1\text{--}10 \mu\text{M}$) and vigorously mixed (1200 rpm, 37°C , 5 min). At the end of this period blood was incubated with fluorochrome-conjugated antibodies and analysed by flow cytometry imaging (ImagestreamX, Amnis).

Results: Stimulation of whole blood with agonists caused concentration-dependent reductions in the population of single platelets that were associated with the appearance of a range of physiological phenomena including platelet microaggregates, platelet aggregates, and platelet-leukocyte interactions with concurrent p-selectin expression or active GPIIb/IIIa.

Conclusion: This approach allows for the investigation of platelet aggregation and platelet-cell interactions in whole blood from humans or experimental animals. Importantly, the low volume permits the simultaneous testing of a wide range of stimulatory conditions. The ease with which this assay can be used to study mouse whole blood allows the ready functional testing of genetic manipulations to better characterise cell to cell interactions in blood.

Disclosure of Interest: None declared.

PO415-TUE

Characterisation of procoagulant platelets in whole blood using a novel cell death marker

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Background: Procoagulant platelets (PP) are implicated in the pathogenesis of thrombotic disorders including coronary events, ischaemic stroke and sepsis outcomes. Our understanding of this platelet subset has been hampered by the lack of a suitable assay for clinical studies. We have previously reported that a novel cell death marker (GSAO) identifies the PP fraction in washed human platelets.

Aims: We developed a flow cytometry-based, non red cell-lyse assay for detection of PP in whole blood suitable for use in human clinical studies and murine models.

Methods: Citrated blood was diluted with buffer containing GPRP, CaCl_2 $\pm$ various agonist(s). Incubation was stopped at 10 min; aliquots were stained with GSAO and antibodies against CD62P, CD45, and CD41a. Following fixation and single wash step, samples were analysed on an LSRFortessa flow cytometer.

Results: Human PP were identified as dual positive (CD62P+/GSAO+) events in the CD41a+ platelet gate. Consistent with known features of PP, $<0.5\%$ of platelets showed the procoagulant phenotype at baseline; thrombin increased the procoagulant fraction ($12.1 \pm 4.3\%$) whereas collagen was significantly less potent ($1.2 \pm 0.9\%$). A combination of thrombin and collagen resulted in the maximal, synergistic response ($15.5 \pm 5.8\%$). Using GPVI agonist, CRP, we demonstrated that the collagen effect is predominantly GPVI mediated. The thrombin-induced response was replicated fully by PAR1 agonist TFLR-NH₂, and only partially by PAR4 stimulation with AYPGKF-NH₂ indicating that the thrombin effect is PAR1 mediated. Stimulation with ADP failed to induce any increase in PP subset consistent with the concept that the procoagulant response is induced by strong agonists.

Conclusion: Using this assay, we have investigated the contribution of PP in coronary artery disease and in murine models of sepsis and atherosclerosis. In conclusion, using a novel marker for necrosis (GSAO), we have validated a whole blood based assay for procoagulant platelets applicable to study of a wide range of disease.

Disclosure of Interest: None declared.

Platelet disorders II

PO416-TUE

Natural history of acquired Glanzmann thrombasthenia: a case report

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Background: Acquired Glanzmann thrombasthenia (GT) is a rare bleeding disorder generally caused by anti- $\alpha_{\text{IIb}}\beta_3$ auto-antibodies.

Aims: To report the case of a patient who has progressively developed an acquired GT.

Methods: Biological tests were (i) platelet aggregation to different agonists, (ii) platelet glycoproteins expression (flow cytometry/western-blot), (iii) presence of anti- $\alpha_{\text{IIb}}\beta_3$ antibodies, and (iv) platelet electron microscopy.

Results: The patient is a 50-year-old man with chronic ITP and Crohn's disease. After unsuccessful splenectomy, romiplostim was initiated and rapidly increased his platelet count. However, in July 2013, he suffered from repeated episodes of gastrointestinal bleedings and platelet aggregation studies indicated a moderate global defect. In December 2013, platelet function testing showed much reduced aggregation using all agonists, but normal agglutination with ristocetin. Expression of $\alpha_{IIb}\beta_3$ was markedly reduced (20% of normal) when rationalized with GPIb α (233% of normal). The presence of an anti- $\alpha_{IIb}\beta_3$ antibody was identified and a platelet aggregation mixing study demonstrated inhibition of normal donor platelets with the patient's plasma. Based on these results, an immunosuppressive therapy was started. Unfortunately, in July 2014, platelet surface expression of $\alpha_{IIb}\beta_3$ has further decreased, whereas the GPIb α expression has increased (300% of normal). Moreover, there were nearly no activation of these receptors or P-selectin expression upon platelet stimulation. Then, a defect in signalling is to be considered in addition to the inhibitory effect of the anti- $\alpha_{IIb}\beta_3$ auto-antibody. Otherwise, the dramatic increase of GPIb α could be explained by the excessive presence of giant platelets seen in the patient, suggesting that these antibodies have also an effect on platelet production.

Conclusion: In conclusion, we present the case of a patient with a severe defect of platelet functions associated with the presence of an auto-antibody directed against $\alpha_{IIb}\beta_3$.

Disclosure of Interest: None declared.

PO417-TUE

Hot-water extracts of the mushroom, *agaricus brasiliensis*, inhibits platelet activation via P2Y₁ receptor

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Background: Some edible mushrooms have inhibitory effects on platelet aggregation. However, the mechanisms which underlie these inhibitory effects have remained unclear. Preliminary experiments using 8 edible mushrooms, we found that hot-water extracts of *Agaricus brasiliensis* specifically inhibited ADP-induced platelet aggregation.

Aims: We report on the mechanism by which hot-water extracts of *Agaricus brasiliensis* inhibit ADP-induced platelet aggregation, and we also explored the possibility of *in vivo* platelet inhibition by oral administration of the mushroom extracts in the mouse model.

Methods: *Agaricus brasiliensis* powder (fruiting body) was extracted with distilled water at 90 °C for 2 h. The extract was collected and freeze dried. The residue dissolved in saline was used for the experiments.

Results: Hot-water extracts of *Agaricus brasiliensis* potently inhibited platelet aggregation induced by ADP, and weakly suppressed platelet aggregation induced by collagen and thrombin-receptor-activating peptide, at the final concentration of 0.5 mg/mL⁻¹. The extracts also had a significant inhibitory activity on platelets shape change and DAMI cells intracellular calcium mobilization induced by ADP via inhibition of ADP binding to the P2Y₁ receptor. Additionally, oral administration of the extracts resulted in prolonged tail bleeding time in mice, with no significant difference in platelet counts between the control mice and high dosage mice.

Conclusion: The hot-water extract of *Agaricus brasiliensis* has inhibitory activities against platelet aggregation via the P2Y₁ receptor. The marked anti-platelet activity of the mushroom extracts involving the P2Y₁ receptor suggests its potential therapeutic use against vascular disorders.

Disclosure of Interest: None declared.

PO418-TUE

A reduced response to TRAP-6 in platelet aggregation studies is not usually due to a PAR-1 receptor defect

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Background: Diagnosing mild bleeding disorders can be challenging as many patients with a mild bleeding phenotype lack a definite diagnosis even after repeated laboratory testing. Also, the clinical significance of certain test results can be unclear. Mild platelet defects may account for many mild bleeding disorders and platelet aggregation studies are undertaken to try and confirm the precise nature of any defect. One of the more common findings on platelet aggregation studies is a reduced response to platelet activation with TRAP-6, which activates the G-coupled receptor, protease activated receptor-1 (PAR-1) encoded by *F2R*.

Aims: We undertook molecular genetic analysis of *F2R* in 8 patients with an abnormal response to TRAP-6 to see if we could identify any pathogenic mutations that might be responsible for the impaired response.

Methods: Both exons of *F2R* were PCR amplified and then sequenced, the large exon 2 being amplified in 3 overlapping fragments. Sequencing was performed on an ABI 3130xl analyser, with sequences analysed using Mutation Surveyor software and compared against the Ensembl reference sequence (ENSG00000181104).

Results: Eight patients with a clearly abnormal response to TRAP-6 on platelet aggregation studies had their *F2R* gene sequenced. In all cases no putative pathogenic mutation was identified. The *F2RL3* gene, which codes for the PAR-4 receptor, was also sequenced in all cases with no putative pathogenic mutation identified.

Conclusion: Platelet aggregation studies suggested impaired thrombin-mediated platelet aggregation in a cohort of patients being investigated for a bleeding phenotype. It might be expected that the PAR-1 receptor, target of TRAP-6, would be the prime candidate for a pathogenic mutation. However, our experience with 8 patients and a literature search suggests that mutations in *F2R* are rare. The cause of the impaired TRAP-6 response in these patients has yet to be identified but is probably in the G Protein-mediated signalling pathway downstream of the PAR-1 receptor.

Disclosure of Interest: None declared.

PO419-TUE

Differential regulation of the platelet proteome in Glanzmann thrombasthenia type I

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Background: Glanzmann thrombasthenia (GT) is one of the best characterized inherited platelet function disorder. Platelets from patients with GT do not aggregate in response to all platelet agonists due to lacking or dysfunctional glycoprotein (GP) IIb/IIIa. However, alterations in the global platelet proteome have not been quantified in these patients so far. Recently, we conducted the first comprehensive and

quantitative characterization of the human platelet proteome (Burkhardt et al. (2012) Blood).

Aims: As proof of concept we aimed to investigate the proteome and function of platelets from a patient with type I GT, caused by a homozygous *ITGA2b* c.175delG frameshift mutation, associated with mucocutaneous bleeding and from four family members without bleeding.

Methods: The global proteome of highly purified washed platelets was quantified by liquid chromatography-mass spectrometry (LC-MS) and targeted MS-methods. Light transmission aggregometry and flow cytometric analysis of platelet surface receptors, fibrinogen binding and CD62P-, CD63-surface expression were performed in platelet-rich plasma according to standardized protocols.

Results: Proteome and flow cytometric analysis confirmed <5% of GPIIb in patient's and about 50% of GPIIb in heterozygotes' platelets compared to unaffected sister. GPIIIa as well as fibrinogen chains were down-regulated up to 10% and 50% in platelets from patient and heterozygotes, respectively. Patient's platelets did not aggregate or bind soluble fibrinogen but showed normal granule secretion capacity in response to platelet agonists. Primary platelet function from heterozygotes and from unaffected sister was in the normal range.

Conclusion: Our data show for the first time the quantitative proteome of a Glanzmann family with the index patient, three heterozygotes and an unaffected individual. This study establishes the utility of LC-MS-based quantitative platelet proteomics as diagnostic tool for inherited platelet function disorders.

Disclosure of Interest: None declared.

PO420-TUE

Granules secretion in patients with Glanzmann's thrombasthenia

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Background: Patients with Glanzmann thrombasthenia (GT) often suffer from severe bleeding complications in case of injury or surgery. Prolonged bleeding time is caused by the diminished or lacking glycoprotein (GP) IIb-IIIa, which mediates platelet aggregation via fibrinogen. However, limited information is available regarding platelet granules release (alpha- and dense bodies) in GT patients. Therefore, we investigated platelet granule release in 10 GT patients.

Aims: The aim was to investigate platelet granule secretion in Glanzmann thrombasthenia.

Methods: Platelet integrin expression and agonist induced PAC1-expression on the platelets' surface was investigated by flow cytometry using monoclonal fluorescence-labeled antibodies (anti-CD41/CD61 and PAC-1). Granules content of the platelets was investigated by using mab's anti-CD63 and anti-CD62P in flow cytometry after platelet activation with thrombin.

Results: FACS analysis of platelet granules using monoclonal antibodies anti-CD62P and anti-CD63 showed an enhanced expression of CD63 ($149 \pm 32\%$) but normal CD62P expression ($83 \pm 24\%$) after platelet activation with thrombin compared to healthy volunteers. GPIIb-IIIa surface expression was reduced to $47 \pm 32\%$. PAC1-expression in response to ADP and TRAP was reduced to $27 \pm 17\%$ and $12 \pm 9\%$, respectively.

Conclusion: The data indicates that GT patients show a higher expression rate of CD63 compared to healthy volunteers. This could be an indication for compensating the lack of GPIIb-IIIa receptor signalling. However, due to the low prevalence of GT further investigation is needed.

Disclosure of Interest: None declared.

PO421-TUE

The *GFI1B*, c.503G>T mutation in the first zinc finger domain predicts a milder bleeding phenotype than the *GFI1B* c.880-881insC mutation

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Background: Inherited *GFI1B* c.880-881insC mutation causes macrothrombocytopenia with a platelet function defect and bleeding tendency; associated with reduced platelet α -granules and red cell anisopoikilocytosis. The phenotype of the *GFI1B* c.503G>T mutation has not been characterised.

Aims: To compare and contrast the clinical phenotypes of *GFI1B*, c.503G>T and c.880-881insC mutations.

Methods: Two unrelated families ($n = 7$) with the *GFI1B* c.503G>T mutation were analysed and directly compared to our previously described family with the c.880-1881insC mutation using the ISTH-SSC bleeding assessment tool and laboratory data.

Results: Both the c.503G>T and c.880-881insC mutations demonstrate autosomal dominant inheritance. The mean bleeding score of individuals with c.503G>T mutations was significantly less than those with c.880-881insC mutations (0.4 vs 3.8 , $P = 0.02$). Both mutations showed large platelets on the blood film and moderate thrombocytopenia. Platelets from c.503G>T cases appeared well granulated and their red cells had normal morphology. Surface glycoprotein expression was normal for both mutations. Whole blood aggregometry in individuals with c.503G>T mutations lacked significant abnormalities, whereas, impaired platelet responses were seen by light transmission aggregometry, in particular to collagen, in individuals with c.880-881insC mutations. Luciferase experiments suggest that the c.503G>T variant has less effect on gene transcription than the c.880-881insC mutation.

Conclusion: The location of *GFI1B* mutation appears to have important phenotypic consequences with the c.503G>T mutation altering the non-DNA binding first zinc finger domain producing a milder phenotype of thrombocytopenia without associated aggregation defects or granule deficiency.

Disclosure of Interest: None declared.

PO422-TUE

Abnormal proplatelet formation and emperipolesis in cultured megakaryocytes form gray platelet syndrome patients

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Background: Gray platelet syndrome (GPS) is an inherited bleeding disorder characterized by lack of platelet α -granules, macrothrombo-

cytopenia and marrow fibrosis. Autosomal recessive form of GPS has been linked to loss of function mutations in *NBEAL2*. Three different *Nbeal2* knock out mouse models were generated and megakaryocyte (MK) and platelet functional and structural features were extensively studied. Mouse models of GPS, however, presented some discrepancies with regard to megakaryopoiesis, proplatelet formation (PPF) and content of MK a-granules.

Aims: We evaluated the *ex vivo* MK development and PPF in 4 patients with GPS and *NBEAL2* biallelic mutations.

Methods: We obtained *ex vivo* cultured MKs from peripheral blood (PB) or bone marrow (BM) hemopoietic progenitor cells (HPC) of 4 GPS patients. CD45 + (PB) or CD34 + (BM) purified cells were cultured for 14 days in the presence of TPO, IL-6 and IL-11. Differentiation, maturation and ploidy of HPC-derived MKs were evaluated by morphological analysis, cytofluorimetric measurement of major membrane glycoproteins and of DNA content, respectively. Adhesion and PPF on fibrinogen and type I collagen coated coverslips were measured as % of adherent cells and % of PPF MKs. The content of a-granule vWF, TSP-1 and P-selectin was evaluated by immunofluorescence analysis.

Results: *Ex vivo* cultured MKs from GPS patients showed both dramatic reduction of the number of proplatelets and gross abnormalities of the shape and ramification of tips. On the contrary, MK development, maturation and differentiation were similar to control MKs. Leukocytes in the MK cytoplasm were found, in agreement with extensive emperipoiesis (36-65% of MKs) in the BM biopsy of GPS patients. The content of vWF, TSP-1 and P-selectin was markedly reduced.

Conclusion: These results suggest that impairment of PPF may be responsible for the thrombocytopenia in GPS patients. Emperipoiesis is a MK-dependent typical feature of the disease. Cultured GPS MKs have reduced content of a-granule proteins.

Disclosure of Interest: None declared.

PO423-TUE

Multiplex phosphospecific flow cytometry for the screening of platelet signaling disorders

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Background: Inherited disorders of platelet signaling are rarely reported due to the lack of appropriate tool for rapid screening in clinical practice. Assessment of intraplatelet protein phosphorylation through flow cytometry would provide this opportunity.

Aims: To develop a rapid and sensitive assay for the detection of platelet signaling defects.

Methods: We screened 4 key phosphoproteins, Akt, LimK, P38-MAPK and SLP76, involved in platelet activation downstream of PAR1, P2Y12, TP α , and GpVI. Citrated platelet rich plasma was incubated for 5 and 15 min in non-aggregating conditions with buffer (base) or thrombin receptor activating peptide, adenosine diphosphate, U46619 or the collagen-related peptide before fixation and permeabilisation. After platelet labelling with a mix of barcoding dyes Alexa[®] fluor 488 and 405 in variable proportions, platelet preparations were pooled and incubated with primary mAbs against phosphoproteins. Secondary antibody conjugated to Alexa[®] fluor 647 was added before analysis by flow cytometry (BD LSR Fortessa[®]). Platelets were gated on their scatter properties and 5.10⁵ events were analysed per sample for median fluorescence intensity (MFI).

Results: Reference values were established in 30 healthy donors. A characteristic profile with the 4 phosphoproteins was established for each agonist. Selected signaling proteins were phosphorylated, but at different extent, downstream of all receptors, except SLP76, specifi-

cally phosphorylated downstream of GpVI. MFI ratios (activated/base) varied between 2 and 4. Specificity was validated by pharmacological inhibitors and analysis of patients with defective receptors. We currently apply the technique to patients with family bleeding history and unidentified thrombopathy.

Conclusion: The multiplex technique allows a rapid and quantitative assessment of platelet signaling on a small volume of blood (5 ml), providing a tool for orienting investigations by biochemical and molecular approaches.

Disclosure of Interest: None declared.

PO424-TUE

Thioredoxin reductase protects platelets from cell death

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Background: Thioredoxin reductase (TRXR) is an important part of the cellular antioxidant defences that maintain intracellular proteins in their reduced state. Auranofin, a gold-based TRXR inhibitor, has been previously used in arthritis. Recently auranofin and other inhibitors of the thioredoxin system have been proposed as novel anti-cancer therapies.

Aims: To determine whether TRXR is an important platelet antioxidant defence.

Methods: Phosphatidylserine exposure in washed platelets was detected by annexin V binding. Intracellular ROS and Ca²⁺ were monitored by CM-H₂DCFDA and fluo-2 fluorescence, respectively.

Results: TRXR activity was detected in unstimulated platelets and could be completely inhibited by auranofin. Following prolonged treatment with auranofin, platelets showed several features of cell death, including an inability to aggregate in response to thrombin, leakage of lactate dehydrogenase, and surface exposure of phosphatidylserine. Auranofin increased platelet reactive oxygen species production and intracellular calcium concentration. DTT, a sulfhydryl reducing agent, and BAPTA-AM, which chelates intracellular calcium, prevented auranofin-induced phosphatidylserine exposure.

Conclusion: TRXR is an important antioxidant defence in unstimulated platelets. TRXR inhibition by auranofin triggers oxidative stress and disrupts intracellular calcium homeostasis, leading to platelet death. There is increasing interest in the development of inhibitors of the TRXR-TRX system in new clinical situations, for example as novel anti-cancer agents. However, the important role of TRXR in protecting platelets means that disruption of this system could lead to unwanted side-effects in platelet count or function.

Disclosure of Interest: None declared.

PO425-TUE

A novel mutation in NBEAL2 gene causing gray platelet syndrome

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Background: Gray platelet syndrome (GPS) is a rare inherited bleeding diathesis characterized by large dysfunctional, agranular platelets that appear gray on Wright-stained peripheral blood smears. The *NBEAL2* and *GFI1B* have been identified to be the causal genes of GPS. Up to date, there were a few articles had been reported.

Aims: To investigate the clinical characters of a GPS patient and explore her molecular mechanisms.

Methods: The morphology of platelets was analyzed by light and transmission electron microscopy. Platelet aggregation was studied on a turbidometric platelet aggregometer (Chronolog) and the expression of platelet membrane GPs was investigated by flow cytometry. Exome sequencing analysis and Sanger analysis of NBEAL2 gene were performed.

Results: The patient was a 22-year-old female and suffered from severe bleeding episodes and thrombocytopenia. Her parents are consanguineous marriage and there was no similar history in her family members. The patient's peripheral blood smear showed large platelets (about 3–5 μm in diameter) with a complete absence of granules, and electron microscopy demonstrated absence of α -granules and her patients' showed variable size of blood platelets with normal granules. Platelet aggregation studies showed lower responses to ADP, epinephrine, collagen, thrombin and U46619, but normal to Ristocetin. The expression of GPIb, GPIIb and GPIIIa on the patient's platelet surface was normal even higher than healthy control. Exome sequencing analysis of the patient identified a C→A homozygous nonsense mutation at position 27713 in NBEAL2 that introduced a stop codon (E1726ter). The pathogenetic mutation were confirmed by Sanger analysis of NBEAL2 gene and her parents and her two relatives were confirmed to be heterozygous for the mutation.

Conclusion: We reported a GPS patient and identified a new homozygous C27713A mutation in NBEAL2 gene leading to E1726ter.

Disclosure of Interest: None declared.

Platelet disorders, inherited / acquired II

PO426-TUE

Validation of the Self-PBQ (self-administered pediatric bleeding questionnaire) as a screening tool for a new diagnosis of Von Willebrand Disease (VWD)

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Background: Expert-administered pediatric bleeding assessment tools have been developed and prospectively validated for the diagnosis of VWD.

Aims: To validate a self-/parent-administered PBQ as a screening tool for a new diagnosis of VWD.

Methods: Children referred to a pediatric hematologist, for the first time, for symptoms of bleeding or bruising, and/or a positive family history of an inherited bleeding disorder (IBD), and/or prior to procedures or surgery for the investigation of a possible bleeding disorder were eligible.

Children were excluded if they had a known diagnosis of an IBD or if there was evidence of an acquired bleeding disorder such as immune thrombocytopenia, hepatic or renal dysfunction, or problems with English or literacy were identified. The study was approved by each of the local ethics committees, and after informed consent/assent was obtained at the initial clinic visit, subjects completed the Self-PBQ prior to meeting with their hematologist.

Hemostatic testing was performed regardless of the resulting bleeding score (BS) (positive BS $\geq$ 2).

Results: To date, 131 subjects (68% female) have been recruited from 5 bleeding disorder clinics, with a mean age of 10 years (range 0–18), VWF:Ag of 0.84 IU mL⁻¹ (range 0.12–2.13), VWF:RCO of 0.77 IU mL⁻¹ (range 0.03–2.22) and FVIII level of 0.98 (0.01–2.57).

The most common reported symptoms include epistaxis, bruising, oral cavity bleeding, and bleeding after surgery.

Hemostatic testing revealed 16 VWD diagnoses (median BS 5, range 0–10), and 14 other IBD diagnoses (median BS 5, range 0–10); testing was normal in 89 children (median BS 4, range 0–15) and 12 are still under investigation. Therefore, for VWD, the Self-PBQ has a sensitivity = 88%, specificity = 40%, PPV = 0.21, and NPV = 0.95.

Conclusion: The Self-PBQ may be a useful screening tool in the evaluation of bleeding symptoms in children presenting for the first time to a hematologist, and will improve clinic efficiency by decreasing the burden of an expert-administered questionnaire.

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PO427-TUE

Family history of venous thromboembolism in the paediatric population: the need for a standardized definition

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Background: Venous thromboembolism (VTE) is an important issue in tertiary care paediatrics. A positive family history is universally agreed to be an independent risk factor for VTE that may or may not reflect an underlying hereditary disorder. However, there is no clear standardized definition of what constitutes a positive family history.

Aims: We aim to assess current published definitions of positive family history as a risk factor for VTE in the paediatric population. We will ascertain if any consensus exists.

Methods: We conducted a literature review through two major databases PUBMED and EMBASE (1969–2014). Two different search statements were used along with criteria selection for each database to maximize the number of relevant results, giving rise to 399 non-duplicated papers.

Results: of the 399 papers, approximately 50% were not relevant to this study, while the remaining articles demonstrated more than 10 separate definitions on what constitutes a positive family history. Variation in definitions were related to the closeness of kinship (first or second degree relatives), whether thrombosis were provoked or unprovoked, the age of presentation of thrombosis in the kinship, and the type of thrombosis (arterial, venous, CNS, myocardial etc.). Of the definitions, 1st degree relative/s developing VTE at any age whether provoked or unprovoked, was the most commonly described definition.

Conclusion: According to this literature review, the definition of a positive family history is non-standardized amongst current published papers. To enable accurate comparisons across studies and improve clinical risk assessment, we therefore propose the need for a standardized definition of what constitutes a positive family history.

Disclosure of Interest: None declared.

PO428-TUE

Initial dose of enoxaparin in neonates with thrombosisHepner M¹, Sciuccati G¹, Cervio C¹, Pieroni G¹, Annetta SE¹, Fariña D², Frontrouth JP¹, Diaz L¹, Torres AF¹ and Bonduel M¹¹Laboratorio de Hemostasia y Trombosis Servicio de Hematología y Oncología; ²Servicio Neonatología, Hospital de Pediatría Prof. Dr. Juan P. Garrahan, Buenos Aires, Argentina**Background:** Enoxaparin is used in the prophylaxis and treatment of thrombosis in neonates. Although ACCP 2012 guidelines recommend an initial dose of 1.5 mg kg⁻¹ q12 h in patients (pts) <2 month, multiple dose adjustments are required to achieve therapeutic range (TR) (anti-Xa level: 0.5–1.0 U mL⁻¹).**Aims:** To determine the enoxaparin dose required to achieve TR in neonates with thrombosis. To establish whether increasing the initial dose of enoxaparin influenced the time required to achieve the TR without an increase in adverse events.**Methods:** Before and after study. From Jan 2004 to Feb 2010, an exploratory retrospective analysis of 20 pts who were treated with enoxaparin (Clexane®) at an initial dose of 1.5 mg kg⁻¹ q12 h and achieved TR within the neonatal period was carried out.From Mar 2010 to Nov 2014, 41 neonates with thrombosis were consecutively recorded. Based on the retrospective analysis, the initial dose was change to 2.0 mg kg⁻¹ q12 h and was prospectively evaluated.

Pts with chylothorax or renal failure were excluded for both evaluations. Continuous variables are expressed as median (range).

Results: The retrospective analysis included 20 pts (8F/12M), age: 18 day (1–28), gestational age (GE): 38w (34–42), birth weight (BW): 3.2 kg (1.2–4.8), thrombosis: venous (VT): 14, arterial (ArT): 5, auricular (AU): 1, dose required to achieve TR: 1.9 mg kg⁻¹ q12 h (1.5–3.2), anti-Xa levels at the first control: 0.24 U mL⁻¹ (<0.1–1.0), time to TR: 3 days (1–7).After intervention, 29/41 pts who met the criteria were analyzed: 11F/18M, age: 18 day (3–28), GE: 38w (34–42), BW: 3.0 kg (1.4–4.4), VT: 18, ArT: 6, AU: 5; dose required to achieve TR: 2.4 mg kg⁻¹ q12 h (1.7–3.6), anti-Xa levels at the first control: 0.44 U mL⁻¹ (<0.1–0.74), time to TR: 1 day (1–3) ($P = 0.0776$).**Conclusion:** Most of the neonates required a higher initial dose of enoxaparin than the currently recommended.Increasing the initial dose to 2.0 mg kg⁻¹ q12 h allowed the reduction of both the time taken to achieve TR and the number of vascular punctures needed to adjust the dose. No major bleeding events were observed.**Disclosure of Interest:** None declared.

PO429-TUE

Skin necrosis and purpura fulminans in children with and without thrombophilia – a tertiary center's experienceFruchtman Y¹, Strauss T², Rubinstein M³, Harosh MB⁴, Revel-Vilk S⁵, Kapelushnik J⁴, Paret G³ and Kenet G⁶¹Pediatric Emergency Department, Soroka Medical Center, Beer Sheva; ²Thrombosis Unit, National Hemophilia Center, Neonatology Department, Safra Children's Hospital, Sheba Medical Center, Tel Hashomer, affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; ³Department of Critical Care, Safra Children's Hospital, Tel Hashomer, affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, Ramat Gan; ⁴The Pediatric Hematology/Oncology department, Soroka Medical Center, Beer Sheva; ⁵The Pediatric Hematology/Oncology department, Hadassah Hebrew University Medical Center, Jerusalem; ⁶Thrombosis Unit, National Hemophilia Center, Tel Hashomer, affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, Ramat Gan, Israel**Background:** Purpura fulminans (PF) is a very rare clinicopathologic skin disorder comprised of dermal microvascular thrombosis associated with perivascular hemorrhage of multiple origin. It may occur as the presenting symptom of severe congenital deficiency of protein C (PC) or protein S (PS) during the newborn period, or later in life following oral anticoagulant therapy with vitamin K antagonists, or of sepsis that may be associated with disseminated intravascular coagulation. Treatment consists of anticoagulants and PC concentrates during acute episodes. We report our experience in the diagnosis and management of pediatric PF.**Aims:** We report our experience in the diagnosis and management of pediatric PF.**Methods:** The medical records of the six children aged 2–16 years (median 5 years) who presented with PF to our tertiary care center between 1996 and 2013 were studied.**Results:** The thrombophilia workup revealed either the presence of congenital homozygous PC deficiency, pro-thrombotic polymorphisms (Factor V Leiden and FIIIG20210A heterozygosity), acquired PC/PS deficiency, or no discernible thrombophilia. The skin necrosis resolved following conservative fresh-frozen plasma/anticoagulant therapy in two cases, while three children required interventional plastic surgery. The sixth case, a 10-year-old child with severe PC deficiency, heterozygous factor V Leiden and FIIIG20210A, received recombinant activated protein C.**Conclusion:** PF in childhood is rare and has multiple etiologies. Understanding of the variable pathogenesis and risk factors will facilitate diagnosis and appropriate clinical management.**Disclosure of Interest:** None declared.

PO430-TUE

Incidence rate and risk factors of venous thromboembolism in Asian pediatric oncology patientsOng WMM¹, Foo KM¹, Lam JCM² and Loke MY¹¹Pharmacy; ²Pediatric Hematology/Oncology Service, KK Women's and Children's Hospital, Singapore, Singapore**Background:** Venous thromboembolism (VTE) is associated with mortality and significant morbidity in the pediatric population. Cancer is a known predisposing factor of VTE, with acute lymphoblastic leukemia (ALL) being the most reported. Currently, data is lacking on the incidence rate and risk factors of VTE in the Asian pediatric oncology population.

Aims: This study aims to evaluate the incidence rate and potential risk factors for the development of VTE in the Asian pediatric oncology population in KK Women's and Children's Hospital (KKH), Singapore.

Methods: This retrospective, single institutional, cohort study was performed in patients with cancer between ages 0–18 years diagnosed and treated in KKH from January 2007 to November 2014. The patient population was identified from the Singapore Childhood Cancer Registry. Patients who had VTE or received anticoagulant therapy prior to cancer diagnosis and those with tumor thrombus were excluded. Demographics and clinical data of patients were retrieved from electronic databases. The risk factors of VTE for all patients and for the ALL subgroup were determined using univariate analyses.

Results: Out of the 837 patients reviewed, 21 patients had VTE, which accounts for a cumulative incidence rate of 2.5%. Among the 199 ALL patients, 10 developed VTE (cumulative incidence rate of 5.0%). The age group of 10 to ≤18 years was found to be associated with the development of VTE with an odds ratio of 7.86 (95% CI = 3.00–20.5) and 13.4 (95% CI = 3.27–55.0) in the cohort and in the ALL patient subgroup, respectively. Other patient demographics such as gender and ethnicity were not associated with VTE development. All of the ALL patients with VTE received *E. coli* asparaginase and 90.0% developed VTE during induction phase.

Conclusion: Incidence rate of VTE in pediatric oncology patients in KKH is low. The age group of 10 to ≤18 years was identified to be a potential risk factor of VTE in our Asian pediatric oncology cohort, as well as in our ALL patient subgroup.

Disclosure of Interest: None declared.

PO431-TUE

Clinical features, risk factors and outcome of pulmonary embolism in children admitted to a single academic center in São Paulo – Brazil: lots to learn or ready to teach?

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Background: Venous thromboembolism (VTE) is a morbid and lethal complication reported in pediatric tertiary centers (PTC) of developed countries. Pulmonary embolism (PE) is considered rare in children, being under-recognized and seldom suspected by pediatricians.

Aims: To describe the epidemiology of children diagnosed with PE at a PTC of a developing country.

Methods: A retrospective review of patients treated between January/2012 and December/2014 including: demographics, presenting symptoms, risk factors, treatment and outcomes was conducted.

Results: Nine children (5 males) aged 11 days to 16.9 years (median age: 13.8 years [CI95%: 7.2–16.8y]) were identified (incidence: 6.0/10,000 admissions). Three patients (30%) also had deep vein thrombosis. Symptoms comprised shortness of breath (78%), pleuritic chest pain (33%) and acute cardiovascular collapse (22%). Underlying diseases included nephrotic syndrome (22%), malignancy (22%), arterio-venous malformation (11%) and myopathy (11%). Risk factors were immobility (44%), infection (44%), catheter (44%), recent surgery (22%) and obesity (11%). Seven patients (78%) had ≥ 2 risk factors for VTE. Diagnostic imaging modalities included computerized tomography pulmonary angiography (67%), echocardiography (22%) and magnetic resonance angiography (11%). Seven patients (78%) were treated with enoxaparin without hemorrhagic complications. One treated patient died of progressive cardio-respiratory failure. Two patients did not receive antithrombotic treatment due to their critical clinical condition and died.

Conclusion: Shortness of breath, underlying disease, immobility, infection, catheter and recent surgery are important clues for early recognition of PE in children with the aim of reducing mortality. In addition our data reinforce the urgent need for studies to implement risk-based guidelines for thromboprophylaxis in hospitalized children in order to improve the survival.

Disclosure of Interest: None declared.

PO432-TUE

An international survey regarding management of coagulopathy in children undergoing liver biopsy

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Background: Many children with liver disease need to undergo liver biopsy for diagnostic purposes or in the assessment of known liver disease. Haemostatic disturbances are common in children with liver disease, including reduced levels of both pro- and anticoagulant factors secondary to a diminished synthetic capacity of the liver. Studies focusing on the impact of coagulopathy on the risk of bleeding complications in the context of pediatric liver biopsy are sparse. There are no evidence-based guidelines regarding management of coagulopathy in children undergoing liver biopsy. Our hypothesis is that pediatric patients with coagulopathy and a strong indication for liver biopsy are managed differently in different centers for pediatric hepatology.

Aims: The aim is to study how children with liver disease and coagulopathy are evaluated and if pro-haemostatic treatment is used prior to liver biopsy, in different pediatric hepatology centers in Australia, Europe and North America.

Methods: A survey regarding management of coagulopathy in children with liver disease in the setting of liver biopsy is produced and sent to 15 centers for pediatric hepatology in Australia, Europe and North America.

Results: The results of the survey will provide information on current coagulation testing, cut-off levels for coagulation factors, liver biopsy methods, treatment options and outcome regarding liver biopsy in recognized centers for pediatric hepatology.

Conclusion: This study will provide important background data for the development of new guidelines on management of liver biopsies in children with liver disease and coagulopathy. The study may also identify important regimens regarding coagulation testing and treatment that can be studied in future clinical trials.

Disclosure of Interest: None declared.

PO433-TUE

Evaluation of thrombophilia in children before renal transplant and outcome of prophylactic anticoagulation post-transplant

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Background: Renal graft thrombosis is a serious early complication of renal transplantation in children. In addition acquired and inherited disorders of clotting system may increase the risk of thrombosis. The role of inherited thrombophilia as an independent risk factor in children is controversial and many reports recommend prophylactic anticoagulation.

Aims: To evaluate the presence of thrombophilia and the outcome of anticoagulation post-renal transplant in children.

Methods: From December 2010 to December 2014 children with end stage renal disease were prospectively evaluated for acquired (prior DVT, prior allograft thrombosis, antiphospholipid antibodies) and inherited (antithrombin, protein S or C deficiency; mutations FV G1691A and FII G20210A) risk of thrombosis. Patients were classified as having high risk of thrombosis if presented the acquired or inherited risk factors above. We administered prophylactic post-transplant intravenous heparin (10 U kg⁻¹ h⁻¹) within the first 24 h and observed the impact of this intervention upon the incidence of allograft thrombosis. Statistics: χ^2 -test.

Results: Twenty two children (13 male), median age 9.0 years (range: 3.0–16.6 y; CI95%: 6.4–11.4 y) were studied. All were classified as having high risk of thrombosis – 18 due to prior DVT and 4 due to prior allograft thrombosis. Three patients also presented inherited thrombophilia – 2 PS deficiency and 1 FII G20210A mutation (high risk vs inherited thrombophilia $P = 0.68$). Eighteen received prophylactic heparin without thrombosis, 4 did not receive heparin due to surgical reasons and 1 patient without prophylaxis had allograft thrombosis ($P < 0.03$). One patient had severe bleeding resulting of an unintentional high dosage of heparin.

Conclusion: The diagnosis of inherited thrombophilia did not impact the risk classification or the outcome in this cohort of patients. Long-term allograft function has been achieved in all patients that received prophylaxis with heparin.

Disclosure of Interest: None declared.

PO434-TUE

Determining the usability of the pediatric thrombosis educational tool (P-TET) in children receiving anticoagulation therapy

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Background: Venous thrombosis is fast growing in children. Despite its increasing prevalence worldwide, there is still a lack of awareness on how to care for children on anticoagulants, particularly where a pediatric anticoagulation (AC) clinic is missing.

Aims: To develop an easy to use and understand bilingual (English, Portuguese) pediatric thrombosis educational tool (PTET) for children on AC for ≥ 3 months.

Methods: PTET included 21 cards with short corresponding written explanations for children grouped into 5 domains (vascular anatomy, clotting process, recognition of clotting events, AC management, and AC side-effects). Content validity was tested in 5 iterative cycles conducted in 2 tertiary centers [São Paulo, Brazil (BR, initial 3 cycles) and Toronto, Canada (CA, last 2 cycles)]. Ease of understanding for visual (V)/written (W) content was evaluated in a 0–5 Likert scale. Time to complete PTET was compared among cycles within countries and between countries (Kruskal-Wallis or Wilcoxon test, as appropriate), and correlated to AC duration (Spearman r). Ethics approval and informed consents were obtained.

Results: Thirty children (BR: $n = 15$, CA: $n = 15$) were recruited (median age: 10.5 years, IQR: 3.7; median AC duration: 14.4 months; IQR: 51). Overall, the mean V/W understanding scores were 4.43 and 4.81 (BR V/W 4.43/4.81; CA 4.43/4.82). Time (minutes) to complete V/W evaluations varied by cycle (BR_V 1st 41.5/2nd 50/3rd 40, $P = 0.23$; CA_V 4th 12.5/5th 10, $P = 0.24$; BR_W 26.5/22/19, $P = 0.03$; CA_W 24/24, $P = 0.72$), and by country (BR_V 42 vs. CA_V 23, $P = 0.001$; BR_W 24 vs. CA_W 24, $P = 0.57$). There was a moderate correlation between time to complete W task and AC duration ($r = 0.44$, $P = 0.018$), but not V task ($r = 0.27$, $P = 0.17$).

Conclusion: Our results confirm the ease of understanding of PTET in children on AC for ≥ 3 months. Subsequent testing will be conducted to evaluate the potential impact on compliance and effectiveness of AC in children, particularly in underserved areas.

Disclosure of Interest: None declared.

PO435-TUE

Rare laboratory presentation of type IIB von Willebrand's disease in a 6 year old boy

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Background: A 6 year old boy was referred to the Coagulation Clinic at Our Lady's Children's Hospital Crumlin, Dublin, Ireland, with a clinical history of bruising.

Aims: To identify the presence or absence of a congenital bleeding disorder in this patient.

Methods: Initial diagnostic laboratory investigations for a suspected congenital bleeding disorder included PT,APTT,fibrinogen and platelet function screening (PFA),FVIII:C,VWF antigen (VWF:Ag), von Willebrand factor activity (VWF:RCO),collagen binding assay (VWF:CB) and full blood count (FBC). Further investigations were subsequently performed on freshly collected samples including repeat VWD screening, ristocetin induced platelet agglutination (RIPA),multimers and VWF genetics studies.

Results: There was no evidence to support a diagnosis of VWD from initial investigations. PT = 12.4 seconds (s) and APTT = 34.4 s with a normal fibrinogen (2.47 g L⁻¹). FVIII:C = 76 iu dL⁻¹, VWF:Ag = 68 iu dL⁻¹, VWF:RCO = 64 iu dL⁻¹ and VWF:CB = 68 iu dL⁻¹. These results were consistent on repeat testing. PFA screening was abnormal COLL/EPI = 144s and COLL/ADP = 160s. RIPA performed at ristocetin concentrations of 1.25 and 0.5 mg mL⁻¹ demonstrated an increased sensitivity to Ristocetin at 0.5 mg mL⁻¹. Mixing tests ruled out platelet type IIB VWD. Multimer analysis was normal. Genetic studies identified a c.3797C>T mutation on exon 28 VWF gene which substitutes Proline by Leucine and is associated with VWD type IIB New York and type IIB Malmö.

Conclusion: A comprehensive repertoire of diagnostic assays routinely performed to identify VWD failed to detect the VWF abnormality in this patient. This case highlights the importance of clinical history in

considering and identifying patients where further investigation may be warranted to elucidate a rare bleeding disorder when an initial extensive diagnostic assessment is normal. This case also illustrates the pivotal role of platelet and genetics studies in the confirmation of type IIB VWD diagnosis in this patient. Mutation analysis is warranted where RIPA is enhanced.

Disclosure of Interest: None declared.

PO436-TUE

Synergistic effect of elevated lipoprotein (a) levels with inherited thrombophilia risk factors in children with arterial ischemic stroke

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Background: Elevated lipoprotein (a) [Lp(a)] has been identified as a genetically determined risk factor for stroke in young adults. Recently, Sultan et al. (Int J Stroke 2014;9:79–87) has also demonstrated a significant positive association between Lp(a) and arterial ischemic stroke (AIS) in children, whereas Kenet et al. (Circulation 2010;21:1838–47) showed the highest odds ratio for combined genetic traits in first AIS onset.

Aims: The aim was to investigate whether elevated Lp(a) levels are more frequently associated with already known inherited thrombophilia risk factors in children with a confirmed diagnosis of AIS.

Methods: Study population comprised 61 children with AIS (perinatal $n = 30$, childhood $n = 31$). Lp(a) concentration was determined using a Tina-quant Lipoprotein(a) assay on Cobas c501 (Roche Diagnostics, Switzerland). Genotype analysis of FV Leiden, FV HR2, FII G20210A, β -fibrinogen –455G>A, FXIII-A Val34Leu, PAI-1 4G/5G, MTHFR C677T, MTHFR A1298C, ACE I/D, and apoE ϵ 2-4 was performed using a multilocus genotyping assay (CVD Strip Assay, ViennaLab, Austria). Human platelet alloantigens –1, –2, –3 and –5 were detected by ASO-PCR (Klütter et al. Vox Sang 1996;71:121–5).

Results: Children with AIS were divided into Lp(a) positive group ($>0.3 \text{ g L}^{-1}$) consisting of 16 (26%) children (median: 0.62 g L^{-1}), and Lp(a) negative group ($<0.3 \text{ g L}^{-1}$) consisting of 45 (74%) children (median: 0.09 g L^{-1}). Elevated Lp(a) levels were statistically significantly associated with homozygosity for MTHFR A1298C ($P = 0.036$) and apo ϵ 2 ϵ 3 genotype ($P = 0.024$). Unexpectedly, none of 7 identified FV Leiden carriers and 3 FV Leiden/FV HR2 heterozygotes were in the Lp(a) positive group, but their frequency was not statistically significant ($P = 0.174$ and $P = 0.556$, respectively).

Conclusion: Among investigated inherited thrombophilia risk factors elevated Lp(a) levels may suggest synergistic effect with MTHFR 1298CC and apo ϵ 2 ϵ 3 genotype and possible increased risk for AIS onset.

Disclosure of Interest: None declared.

PO437-TUE

The role of FVR2 polymorphism in children carriers of FVLeiden

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Background: FVLeiden (FVL) has been identified as a risk factor for thrombosis. Another haplotype of the factor V gene (FVR2) has been considered to interact synergically with FVL to increase the risk. Nevertheless, it has been discussed in literature that the FVR2 polymorphism might have a protective effect for thrombosis.

Aims: The effect of FVR2 in combination with the presence/absence of FVL in children with or without thrombosis.

Methods: The significance of the differences in the distributions of FVL, FVR2 and their combinations among normal controls ($n = 103$) and children with thrombosis ($n = 210$, 60.47% CNS arterial thrombosis) and without thrombosis but with family history of FVL thrombophilia ($n = 170$) were checked with the 95% confidence intervals (CI) of the corresponding odds ratios (OR). All studied samples were processed according to the CVD Strip Assay T, Vienna Lab, Austria manufacture protocol (DNA isolation, PCR amplification, hybridization of amplification products to a test strip containing allele specific oligonucleotide probes).

Results: The proportion of homozygous/heterozygous state of FVL amongst children with thrombosis (13.9%) is significantly higher than among normal controls (4.9%, OR = 3.1, 95% CI 1.8–8.4) and even among children with family history of FVL thrombophilia (7.1%, OR = 2.1, 95% CI 1.0–4.3). Conversely the proportion of the wild type of FVR2 amongst children with thrombosis (89.8%) is significantly higher than among normal controls (82.6%, OR = 0.5, 95% CI 0.2–0.9). A total OR of 5.0 (95% CI 1.6–15.5) of heterozygous/homozygous state of FVL and the wild type of FVR2 in comparison to the wild type of FVL and to the heterozygous/homozygous state of FVR2 was found.

Conclusion: There is an additive risk effect for thrombosis by the coincident presence of homozygous/heterozygous state of FVL and of the wild type of FVR2. Therefore, the FVR2 polymorphism could have a protective role in children with FVL mutation.

Disclosure of Interest: None declared.

PO438-TUE

Whole blood thromboelastometry (ROTEM®) profiles in children with cyanotic congenital heart diseases undergoing cardiac surgery

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Background: Cyanotic heart disease is characterized by a complex dys-coagulopathy that increases both the thrombotic and the hemorrhagic risk in children undergoing cardiac surgery. It is known that the classic coagulation tests are unable to predict the thrombotic or bleeding risk after complex cardiac surgical corrections.

Aims: To evaluate the use of whole blood thromboelastometry (ROTEM®) in children with congenital heart disease (CHD).

Methods: Standard coagulation tests and ROTEM® assays were performed on 81 consecutive paediatric patients who underwent surgery for CHD. 21 (26%) patients were cyanotic and 60 (74%) non-cyanotic. Mean age at surgery was 7.9 months (range 1 day–16 years).

Results: Cyanotic patients had a significantly higher hematocrit ($P < 0.001$), a reduced prothrombin activity (PT %, $P = 0.01$) and

lower platelets count ($P = 0.02$) than non cyanotic patients. An inverse linear association was found between Clot Formation Time (CFT) and SATO₂ (INTEM, $P = 0.001$ and EXTEM, $P < 0.0001$), and also between CFT and preoperative platelets count (INTEM, $P = 0.0005$ and EXTEM, $P = 0.0003$). A direct linear association was found between Maximum Clot Firmness (MCF) and patients' SATO₂ (INTEM, $P = 0.04$ and EXTEM, $P = 0.05$). Preoperative cyanosis was also associated with a lower median MCF in FIBTEM ($P = 0.02$). Cyanotic patients required more frequently postoperative transfusions of fresh frozen plasma ($P = 0.01$) and fibrinogen ($P = 0.0001$). Pre-operative lower PT and platelets count were associated to an increased postoperative fibrinogen administration ($P = 0.02$ and $P = 0.0001$, respectively), a longer CFT (INTEM, $P = 0.03$ and EXTEM, $P = 0.007$) and a reduced MCF (EXTEM, $P = 0.01$ and FIBTEM, $P = 0.002$).

Conclusion: ROTEM® parameters add important specific preoperative information to classic coagulation parameters in patients with CHD. Cyanotic patients presented with preoperative coagulation anomalies significantly requiring higher postoperative blood product supplementation.

Disclosure of Interest: None declared.

PO439-TUE

The incidence of deep vein thrombosis in children with peripherally inserted central catheters versus tunneled centrally inserted catheters in pediatric patients: a prospective cohort study

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Background: The incidence of deep venous thrombosis (DVT) in children has risen largely due to the increased use of central venous catheters (CVC). CVC are placed for venous access in children with serious acute or chronic disorders, however it is not clear if the incidence of CVC-related DVT is impacted by the type of catheter or insertion technique. There are several varieties of CVC, but the two main types are peripherally-inserted central catheters (PICC) and centrally-inserted tunneled lines (TL). Particular characteristics of the CVC, such as insertion site, caliber, and intravascular course may alter the risk for DVT.

Aims: With this IRB-approved, prospective observational cohort study, we aim to compare the DVT incidence with various CVC types, as well as evaluate risk factors (such as catheter insertion techniques and underlying medical issues) for the development of CVC-related DVT.

Methods: Subjects aged 6 months to <18 years, who either had a PICC or TL placed were consented and followed up to 1 year or until a DVT occurred. Data was collected from the electronic health record.

Results: The interim analysis includes 307 subjects with 356 lines. 161 (52%) of subjects were male. The median age was 5 years (IQR 0.5–17.9). There were 13 CVC-related DVT (3.7%), and 12 (92%) were in subjects with PICC. Univariate analysis showed that PICC related DVT had an increased hazards ratio of 8.7 (95% CI, 0.98–76.9, $P = 0.01$). Other significant variables included multiple CVC lumens [HR=3.6 (95% CI, 1.10–11.8), $P = 0.04$], and congenital heart disease [HR = 4.9 (95% CI, 1.3–18.2), $P = 0.04$].

Conclusion: In this interim analysis, subjects with a PICC compared to those with TL had a significantly increased risk for DVT. Other significant risk factors included the number of lumens and a history of congenital heart disease. Continued subject recruitment will occur in order to complete the evaluation of the other proposed risk factors and to conduct a multivariate analysis.

Disclosure of Interest: None declared.

PO440-TUE

Benign joint hypermobility syndrome is prevalent in children with undefined bleeding disorders

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Background: Many children followed in bleeding disorder clinics do not have a defined diagnosis or have bleeding phenotypes with normal test results. Disorders of collagen, presenting as joint hypermobility syndromes, may contribute to bleeding phenotype in children but the prevalence is unclear.

Aims: To apply the Benign Joint Hypermobility Syndrome (BJHS) diagnostic criteria to children with von Willebrand disease (VWD) or undefined bleeding disorders (UBD) and determine the prevalence of hypermobility along with evaluation of joint hypermobility symptoms (Brighton criteria) and its relation to the pediatric bleeding questionnaire scores (PBQ) and laboratory testing.

Methods: Consecutive patients with a diagnosis of VWD or UBD (and/or parents) answered a self-administered questionnaire to determine the presence or absence of features consistent with symptomatic joint hypermobility (Brighton criteria), diagnostic of BJHS. PBQ and joint examination (Beighton Score) were administered. Historical laboratory tests were retrieved from medical records.

Results: 20 patients were enrolled (VWD $n = 11$; VWD T2 $n = 1$; Undefined Platelet Defect $n = 2$; UBD $n = 5$; no bleeding disorder $n = 1$). Median age was 12 years (range, 7–18) with an equal number of males and females. PBQ score was ≥ 2 in 19 patients (median 5.5, range 1–12). Joint hypermobility via the Beighton score (>4) was present in 3 patients, all ≥ 12 years of age. Four (20%) patients met criteria for BJHS, and in those with UBD, 60% met BJHS criteria. BJHS patients were either classified as undefined bleeding disorder ($n = 3$) or undefined bleeding disorder/platelet defect ($n = 1$).

Conclusion: This is the first report of BJHS in a pediatric bleeding disorder cohort. The diagnosis of BJHS in children can be difficult as younger children (<10 years) are recognized to have intrinsically greater joint mobility. However, we found evidence of BJHS in older children with UBD suggesting it is common and should be considered during the diagnostic process.

Disclosure of Interest: None declared.

PO441-TUE

Thromboembolic complication in childhood with nephrotic syndrome: experience in three pediatric hospital in Argentina

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Background: Nephrotic syndrome (NS) is associated with an increased risk for thromboembolic complication. Its incidence in children is about 3%. The pathophysiology of thrombosis in the NS seems to be multifactorial.

Aims: Thrombotic complications in NS and risk factors.

Methods: A descriptive, retrospective study was made on pts diagnosed with NS who had thrombotic complications (TC) in 10 years in 3 pediatric institutions.

Results: 11 pts diagnosed with NS had 13 TC. The median age at diagnosis was 7 years (2–16 years) 73% of them were steroid-dependent, and 64% were male. The locations of the TC were varied: brain ($n = 3$) arteria $n = 1$ venous sinus $n = 2$; lower limbs DVT ($n = 7$), neck veins ($n = 1$) and intracardiac ($n = 2$). Diagnosis was confirmed by color Doppler ultrasound, tomography and MR angiography. 100% of the children had hypoalbuminemia $<2 \text{ g dL}^{-1}$ and proteinuria in the nephrotic range.

Hematologic alterations favoring thrombosis were: thrombocytosis in 55% (mean $427 \times 10^9 \text{ L}^{-1}$), elevated fibrinogen in 9/11 (mean 800 mg dL^{-1}) and antithrombin dosing was performed on 9/11 ATIII deficiency detected in 8/9.

Other associated risk factors were: central catheters (4 pts), Infections (3 pts) also the use of diuretics, dehydration, and parenteral nutrition. In 2 pts (1 pte with CNS and intracardiac thrombosis and 1 pte with tr. Cerebral), were later diagnosed SLE. Thrombotic episodes were initially treated with UFH only in 2 pts and with LMWH in the others, followed with oral anticoagulants (10/11). In a pts with intracardiac thrombosis, thrombectomy was required. 4 ptes received extended prophylaxis for persistent risk factors. 3 ptes received prophylaxis treatment for relapses. There was no recurrence.

Conclusion: In children with NS, a high index of suspicion is required for TC in order to institute an early treatment and get a favorable outcome. The use of antithrombotic prophylaxis during relapses should be individualized according to association with risk factors such as hypoalbuminemia and infections.

Disclosure of Interest: None declared.

PO442-TUE

Acquired von Willebrand Syndrome (AVWS) – an underdiagnosed complication in severely ill pediatric patients with bleeding complications

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Background: Von Willebrand factor is a multimeric protein which is required for platelet adhesion and plays an important role in haemostasis. Generally, acquired von Willebrand syndrome (aVWS) is associated with a recognizable underlying disorder. aVWS is reported with a prevalence of 0.04 to 0.13%. According to the ISTH registry, lymphoproliferative (48%), cardiovascular (21%), myeloproliferative (15%), other neoplastic (5%), and autoimmune disorders (2%) are most common.

Aims: Acquired VWS seems to be an underdiagnosed condition in pediatric patients.

Methods: We report the cases of critical ill paediatric patients with severe bleeding who developed aVWS. Underlying disorders included dyskeratosis congenita (two boys), posttransplantation lymphoproliferative disease (one girl), sepsis with multiorgan failure (two patients), congenital heart failure with stenosis (3 boys), unknown immunodeficiency (one girl), and progressive familial intrahepatic cholestasis (one boy).

Results: In most cases the treatment of bleeding was very difficult.

Conclusion: Acquired VWS seems to be an underdiagnosed condition in critical ill paediatric patients. The most common causes of avWF in children are cardiac valvular disease, hypothyroidism, and Wilm's tumor. Uraemia, lymphoma, microangiopathy can be further conditions. Therefore, if there is unexpected bleeding in a critically ill pediatric patient, aVWS should be suspected. Specific treatment can be a challenge, however.

Disclosure of Interest: None declared.

PO443-TUE

The impact of serum bile acid levels on the mRNA expression of pro-and anticoagulant proteins in liver tissue

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Background: Coagulation factors are used as prognostic markers in liver disease, i.e. the levels are expected to decrease as the liver function deteriorates. However, in cholestatic liver disease, increased rather than decreased levels of coagulation factors have been demonstrated in patients with highly elevated levels of bile acids. The mechanism for this paradoxical finding is unclear and these effects are overlooked when coagulation factors are used as prognostic markers in patients with cholestatic liver disease.

Aims: To investigate if serum bile acid levels have an impact on the mRNA expression of pro-and anticoagulant proteins in hepatocytes.

Methods: Six patients (0.5–9.8 years) with progressive familial intrahepatic cholestasis (PFIC), who consecutively underwent the surgical procedure partial external biliary diversion (PEBD) to reduce high serum levels of bile acids, were enrolled in this cohort study at our tertiary referral center for pediatric hepatology. Informed consent was obtained and the study was approved by the ethics committee. INR, total fasting serum bile acids and liver biopsies were collected before and after the procedure. Eight age-matched non-cholestatic patients with α -1 antitrypsin deficiency, undergoing liver biopsy, served as controls. mRNA specific for coagulation factor II (FII), FV, FVII, fibrinogen, protein C and Farnesoid X receptor (FXR) were extracted and analyzed for each liver biopsy.

Results: The mRNA levels of Factor V, Factor VII, the three fibrinogen chains and protein C were significantly higher compared to controls, prior to PEBD. A reduction in bile acids levels from median $298 \mu\text{mol L}^{-1}$ (range 83 – $687 \mu\text{mol L}^{-1}$) to $4 \mu\text{mol L}^{-1}$ (range 2 – $145 \mu\text{mol L}^{-1}$) after PEBD was seen. The mRNA levels after the procedure did not differ from controls, except for Factor V-mRNA which remained significantly higher than in the controls.

Conclusion: High levels of bile acids may stimulate the mRNA expression of both pro-and anticoagulant proteins.

Disclosure of Interest: None declared.

PO444-TUE

Thrombomodulin gen analysis in a case of atypical hemolytic-uremic syndrome

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Background: Atypical haemolytic-uremic syndrome (HUS) presents the classic triad (microangiopathic haemolytic anaemia, thrombocytopenia and renal failure) in absence of secondary cause. Half of the

patients with this form have mutations in genes that regulate the complement system. Mutations in the thrombomodulin gene have been reported in 5% cases.

Aims: A 21 month- old female was admitted to emergency room with 5 days evolution fever. Physical examination revealed micropetechiae at lower limbs and hepatomegalia. Laboratory parameters showed microangiopathic haemolytic anaemia, acute renal failure, thrombocytopenia, hyperferritinemia, hypofibrinogenemia, elevated D-dimer concentration and 9% of ADAMTS-13 activity. Bone marrow examinations disclosed images of hemofagocytosis. With these clinical and laboratory criteria the patient was diagnosed of atypical HUS and macrophagic activation syndrome. The patient was initially treated with immunoglobulins, high dose methylprednisolone and fresh frozen plasma and finally with eculizumab. We observed a progressive recovery until the complete resolution of the episode.

Methods: We investigated thrombomodulin (THBD) mutations associated with the atypical HUS by Delvaeye et al. ABI3500 (Applied Biosystems®) was used to Sanger sequence the regions of the gene delimited by the the following primers: TM1F5'- GACGGAC AGGAGAGGCTGT-3' and TM1R 5'- TGGTGTGTTGTCTCC CGTA-3' (437 bp, used for detecting A43T, D53G, V81 L mutations) and TM2F 5'- GAAGGCTACATCTGGACGA-3' and TM2R 5'- GCAGCACTACCTTGGAA-3' (406 bp, aimed to study P495S, P501L and D486Y mutations).

Results: No mutations were found in the fragments sequenced of the patient. In our patient, DNA fragments from THBD gene analysed by direct sequencing do not show the described variations. We performed the same analysis in a control group without findings.

Conclusion: The patient here discussed did not present described THBD mutations. Next step will be sequencing of the entire THBD gene to characterize candidate new variants.

Disclosure of Interest: None declared.

PO445-TUE

Clinical characterization of venous thromboembolism in childhood at a tertiary level hospital

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Background: Venous thromboembolism (VTE) is rare among children and clinical characteristics and risk factors differ from those of the adult population. In the last few years, incidence has increased substantially due to intensified health care in critically ill patients.

Aims: To describe clinical characteristics and risk factors of VTE in the pediatric population of a tertiary care center.

Methods: A retrospective analysis was performed among children diagnosed with VTE that were referred to the Thrombosis Unit over an 8-year period. Subjects were classified in three groups: neonates (0–2 months), infants (2 month- 2 years) and children (2- 18 years).

Results: A total of 107 patients were included in the analysis. 74% of cases were male and the age group distribution was 20% of neonates, 46% infants and 33% children. When analyzing all age groups the most common clinical presentation was deep venous thrombosis (67%), cerebral sinus thrombosis (17%), intrahepatic (6%), pulmonary embolism (5%) and renal vein (5%). Taking age classification into account, the most frequent location in neonates and infants was the vena cava (34% and 47% of cases respectively), and cerebral sinus thrombosis in almost one-third of children (30%). 78% of events were provoked and the most important risk factor in the neonates and infants group was central venous catheter insertion and congenital heart diseases (69%). Thrombophilia testing was performed in 33 cases (31%), but most of them were not valuable.

Conclusion: As described in the literature, VTE in pediatric population is mostly related with triggering factors and clinical presentation depends on the age group. The increasing number of high complexity procedures in congenital heart diseases and a generalized use of central venous catheter in acute cure units in a tertiary hospital could influence in the high proportion of these patients.

Disclosure of Interest: None declared.

PO446-TUE

Let's cut to the bone: risk factors of venous thromboembolism in children with musculoskeletal infections

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Background: Venous thromboembolism (VTE) has been described in children with musculoskeletal infections (MSI). Yet, VTE predictors and VTE association with adverse MSI outcomes are unknown.

Aims: To identify risk factors of VTE in children with MSI, and to explore associations of VTE with MSI-related outcomes.

Methods: Patients (pts) aged 0–18 years diagnosed with acute (≤ 2 weeks) or subacute/chronic MSI [clinical, imaging, and/or positive cultures (CX+)] between 2006 and 2014 were included. VTE positive (VTE+) cases were objectively confirmed. Data on VTE predictors [clinical: shock, inotrope use, catheter (CVL) presence, CX+; biomarkers: platelet count, erythrocyte sedimentation rate, C-reactive protein (CRP), white blood cell count], and MSI-morbidity (length of stay (LOS), severe functional disturbance (SFD), death), were collected. Ethics approval was obtained.

Results: Overall, 210 pts with MSI, mean (range) age 8.1 (1–18) years, 53% males, were identified. There were 187 (89%) osteomyelitis and 23 (11%) septic arthritis; 65% of pts had CX+ (45% methicillin-sensitive *Staph. aureus*).

VTE occurred in 11% of cases, at a median of 4 (1–184) days after MSI diagnosis, and mostly during acute MSI (87% vs. 13% subacute/chronic, $P = 0.06$). Pelvis/lower limb combined was the most common infection site (65%), similar to VTE distribution [lower limb (65%), upper limb (30%), abdomen (5%)]. Around 40% of VTE+ cases were CVL-related. Multivariable logistic regression showed that CX+ and initial CRP were independent VTE predictors [odds ratio 10.2 (95% CI 1.3–78.3) for CX+ vs. CX–, $P = 0.03$; and 1.08 (95% CI 1.03–1.14) for each $\times 10$ -increase in initial CRP, $P = 0.001$]. Unfavorable MSI outcomes were more commonly identified in patients with VTE [LOS (VTE+ 24 days vs. VTE– 12 days, $P = 0.01$); SFD (VTE+ 22% vs. VTE– 6%, $P = 0.02$); VTE+ 1 death].

Conclusion: VTE occurred in 10% of MSI cases in children, mostly during the acute phase. VTE appears to be a marker of disease severity, and its contributory role on MSI-related outcome needs to be further explored.

Disclosure of Interest: None declared.

PO447-TUE

Feasibility and safety of enoxaparin whole milligram dosing in premature and term neonates

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Background: Neonatal thromboembolism (TE) is commonly treated with enoxaparin. To deliver weight-based dosing of enoxaparin, dilutions are often necessary and can lead to medication errors. Since

2008, all neonates with TE are administered whole milligram doses of enoxaparin via insulin syringe at our center.

Aims: The primary objective was to determine feasibility, defined by the ability to achieve therapeutic anti-factor Xa (anti-Xa) levels (0.5–1.0 u mL⁻¹), and safety, measured by occurrence of bleeding episodes, of whole milligram dosing of enoxaparin in premature and term neonates. The secondary objective was to assess response to treatment.

Methods: Medical records of neonates with TE treated between January, 2008 and December, 2014 were reviewed for demographics, details of TE diagnosis, enoxaparin dosing and adjustments, anti-Xa levels and bleeding episodes.

Results: Nineteen premature (<37 weeks, most premature 23.3 weeks and 530 grams) and 21 term neonates were diagnosed with TE during the study period (Arterial 6, Cardiac 4, CSVT 5, DVT 14, RVT 3, PVT 14). Six patients had >1 TE. The mean starting and therapeutic doses were 1.91 mg kg⁻¹ (premature 2.20 mg kg⁻¹, term 1.64 mg kg⁻¹) and 2.09 mg kg⁻¹ (premature 2.41 mg kg⁻¹, term 1.82 mg kg⁻¹), respectively. Among 39 neonates whose anti-Xa level was checked, 25 (64%) reached therapeutic levels with starting dose, while 14 (36%) required dose adjustments for a mean of 1.6 adjustments per patient. Only 1 term neonate reached initial supratherapeutic anti-Xa level >1.0 u mL⁻¹ (1.23 u mL⁻¹), while 4 premature and 5 term neonates reached >1.0 u mL⁻¹ at one point during anticoagulation. There were no associated bleeding episodes. Neonates received enoxaparin for mean of 12 weeks (12 days to 45 weeks). Among 34 evaluable patients at the end of treatment, 23 (68%) had complete response, 9 (26%) partial and 2 (6%) had stable TE.

Conclusion: Whole milligram dosing of enoxaparin via insulin syringe is feasible, safe and effective in premature and term neonates requiring treatment for TE.

Disclosure of Interest: None declared.

Platelets and cancer

PO448-TUE

Immunophenotypic expression of platelets in myelodysplastic syndrome

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Background: Myelodysplastic syndrome (MDS) is a hematopoietic stem cell-associated bone marrow disorder. Recent studies with the technique of flow cytometry demonstrated an alteration of antigen expression on patients' leukocyte, suggesting that the data regarding the antigen expressed on leukocyte may help the diagnosis of MDS. The platelet count in MDS patients is abnormal as well. Thrombocytopenia is a common manifestation, especially in the severe cases.

Aims: This study aims to find the answer by comparing the platelet surface antigen level between healthy controls and MDS patients.

Methods: Twelve healthy controls and 15 MDS patients were enrolled in this study. The levels of platelet surface antigens, including CD31, CD41a, CD42a, CD42b and CD61, were determined by flow cytometry, reflected by the maximal binding of fluorochrome-conjugated monoclonal antibodies.

Results: The levels of CD41a and CD61 on MDS patients' platelet were shown to be lower than those of the healthy controls. The levels of CD42a and CD42b on the other hand were indistinguishable between both groups. We also found the platelet size of MDS patients is larger than that of healthy controls by flow cytometry. If the content of platelet surface antigens were adjusted based on the platelet size, the level of CD41a, CD61, CD42a and CD42b on the platelet of patients with MDS would be lower than that of healthy controls.

Conclusion: All the results revealed that the patients with MDS have platelets with larger size than healthy controls, but the expression of CD41a, CD61, CD42a and CD42b on platelet surface were lower

those of healthy adults, thus demonstrating a quality change of platelet production in MDS patients. These data may help the diagnosis of MDS and also be useful in the outcome prediction during treatment.

Disclosure of Interest: None declared.

PO449-TUE

Recursive partitioning with amalgamation analysis of cancer specific biomarkers in the prediction of cancer associated thrombosis

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Background: Venous Thromboembolism (VTE) is a leading cause of death among patients with cancer. There is a growing body of data underscoring the importance of platelets in cancer associated thrombosis.

Aims: We sought to evaluate platelet specific biomarkers as predictors of cancer associated thrombosis.

Methods: We performed a prospective sample collection, with a retrospective blinded evaluation. We included outpatient adults with solid tumors planned to receive chemotherapy; patients were excluded if they were pregnant, hospitalized within last 2 weeks, or had an acute VTE. Platelet specific candidate biomarkers included platelet count, collagen-thrombin activated platelets (COAT), mean platelet volume (MPV) and procoagulant microparticles (PPL). VTE and death were periodically adjudicated. We evaluated for potential interactions with age, gender, race. We used JMP for recursive partitioning and amalgamation (RECPAM) to refine the model. Biomarkers were categorized according to quartiles or recursive partitioning suggested levels. We used SAS 9.2 cox proportional hazard analysis using death as competing event.

Results: We analyzed 241 patients: 60 yo (median), 72% female. 13% developed VTE, and 15% died. Median follow up 11.9 months. There was an interaction between age and COAT. In the univariate analysis, platelet count >334000 mL⁻¹ (Hazard Ratio: 2.4; 95%CI 1.1–5.2), COAT >45% in younger patients (less than median age) (2.7; 1–7.1), and MPV <9.8 fL (2.9; 1.2–6.8) were associated with incident VTE. The RECPAM model included high platelet count (3.3; 1.4–8) as first decision point and COAT (3.6; 1.2–10) in younger patients as second. The rest of the patients were amalgamated as reference. High platelet (3.1; 1.2–7.5) and COAT (4.6; 1.6–13.6) in younger patients remained VTE predictors after adjusting for metastatic disease, cancer type and functional status.

Conclusion: COAT platelet levels added to absolute platelet count in the prediction of cancer associated thrombosis.

Disclosure of Interest: A. Tafur Grant/Research Support from: Stago Diagnostica, G. Dale: None declared, A. Mansfield: None declared, M. Cherry: None declared, P. Comp: None declared, S. Rathbun Grant/Research Support from: Stago Diagnostica, J. Stoner: None declared.

PO450-TUE

Cancer cells induce the release of microparticles from human platelets

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Background: Circulating blood platelets are essential to support cancer metastasis. Platelets protect cancer cells from the immune system, and

release bioactive molecules that control metastasis and angiogenesis. In addition, platelet-derived microparticles (PDMPs) may also regulate cancer spread, as increased levels of circulating PDMPs are found in cancer metastatic patients. However, the ability of tumor cells (TC) to directly induce the release PDMPs has never been documented.

Aims: To investigate and characterize the release of PDMPs induced *in vitro* by breast (MDA-MB-231) and colorectal (Caco-2) cancer cell lines.

Methods: Fluorescently labeled platelets in plasma or buffer were incubated with TC for up to 30 minutes, and isolated PDMPs were quantified. TC-induced platelet aggregation (TCIPA) was concomitantly measured by lumiaggregometry.

Results: MDA-MB-231 and Caco-2 cells failed to induce PDMPs release in citrated PRP, but were effective on platelets resuspended in non-anticoagulated serum. For both cell lines, PDMPs release was about the 25% of that produced by thrombin. Similarly, TC stimulate PDMPs release from unwashed isolated platelets suspended in buffer, but not from extensively washed platelets. Addition of a small volume (0.05% v/v) of plasma or serum was sufficient to restore TC-induced PDMPs release from washed platelets. TCIPA was constantly observed only when PDMPs release occurred, and required the presence of small amount of plasma. TCIPA and PDMPs release were reduced by PGE1 or apyrase, indicating that are consequent to platelet activation, and were abrogated by PPACK, suggesting a possible role for thrombin. TCIPA also required either fibrin polymerization or integrin $\alpha_{IIb}\beta_3$ activity.

Conclusion: MDA-MB-231 and Caco-2 stimulate PDMPs release in association to full platelet aggregation. The sole interaction between cancer cells and platelets is not sufficient to trigger these responses, which require the contribution of plasma components.

Disclosure of Interest: None declared.

PO451-TUE

The effects of interferon α -2b on the defective bone marrow mesenchymal stem cells in patients with essential thrombocythemia

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Background: Essential thrombocythemia (ET) is a myeloproliferative neoplasm characterized by a risk of thrombotic and hemorrhagic complications. Bone marrow microenvironment may involve in the pathogenesis of MPN. Bone marrow-derived mesenchymal stem cells (BM-MSCs) are key components of the hematopoietic microenvironment. Interferon alpha (IFN- α) appeared to be a good therapy due to its anti-tumor effects.

Aims: The biological and functional characteristics of MSCs from ET patients in Chinese population are unclear and the effects of IFN- α -2b exerted on BM-MSCs are also needed to be clarified. The aim of this study is to elucidate the above two challenges.

Methods: BM-MSCs from patients with ET ($n = 24$) and age- and sex-matched healthy individuals ($n = 13$) were cultured in appropriate growth medium. The biological and functional characteristics of BM-MSCs with or without exposure to IFN- α -2b from the two groups were determined and compared.

Results: BM-MSCs from the two groups shared similar characteristic, including morphology, immunophenotype, adipogenic differentiation potentials. However, the patients BM-MSCs had a higher proliferation capacity and lower capacity of osteogenic differentiation, apoptosis rate and extent to support hematopoiesis. IFN- α -2b increased apoptosis rate and enhanced patients BM-MSCs to expand CD34+ hemo-

poietic progenitor cells, but deregulated the ability to differentiate into osteoblasts and adipocytes and to produce hematopoietic colony-forming unit – megakaryocyte.

Conclusion: BM-MSCs derived from ET patients were abnormal and might participate in the occurrence of ET. IFN- α -2b is a good candidate for ET patients through affecting the defective BM-MSCs.

Disclosure of Interest: None declared.

PO452-TUE

Sunitinib is taken up by platelets and inhibits platelet activation and thrombus formation in cancer patients

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Background: Sunitinib is an oral receptor tyrosine kinase inhibitor used for treatment of several solid tumors. Patients treated with sunitinib are at higher risk of bleeding (no thrombocytopenia). As protein tyrosine kinases are essential for platelet function, we hypothesized that platelet functional properties are altered in patients treated with sunitinib.

Aims: Investigate the effect of sunitinib on platelet functions *in vitro* and in cancer patients on treatment.

Methods: The study was approved by the medical ethics committee from the Maastricht University Medical Centre. Blood samples from healthy volunteers ($n = 8$) and advanced renal cell carcinoma patients ($n = 8$) before and 2 weeks on-treatment with sunitinib (monotherapy) were collected after informed consent. Platelets were washed and preincubated with sunitinib (3.3–30 μ M) or vehicle for 10 minutes at 37 °C. Flow cytometry, aggregometry, ELISA, immunofluorescence imaging and whole blood perfusion over collagen were performed.

Results: Confocal microscopy indicated sunitinib accumulation in platelet granules. *In vitro*, collagen and ADP-induced platelet aggregation were inhibited by sunitinib in a concentration-dependent manner. In patients treated with sunitinib, glycoprotein VI-induced platelet aggregation as well as activation of integrin $\alpha_{IIb}\beta_3$ and P-selectin expression were reduced compared to controls. Both *in vitro* and in patients, sunitinib reduced collagen-dependent thrombus formation and phosphatidylserine exposure under flow conditions. Secretion of angiogenic factors (PDGF and CTAPIII) upon platelet activation was reduced in the presence of sunitinib. In 4 patients, mild bleeding symptoms were observed that correlated with more reduced thrombus formation.

Conclusion: Sunitinib is taken up by platelets and inhibits collagen receptor-induced integrin activation, secretion, aggregation and thrombus formation. This may contribute to the higher bleeding tendency observed in patients treated with sunitinib.

Disclosure of Interest: None declared.

Regulation of coagulation and fibrinolysis I

PO453-TUE

Allelic variants within ANXA5 differentially impact on the control of gene expression

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Background: In 2007, two haplotypes (M1 and M2) within the ANXA5 promoter were described in women with pregnancy loss. In an *in vitro* functional assay, they showed a significant association with a lower activity of the ANXA5 promoter. The M1 and M2 haplotypes are defined by 4 allelic variants. However, no information is available on the differential impact of each variant on the control of ANXA5 expression.

Aims: To provide information on functionality of 4 allelic variants: c.-467G>A, c.-448A>C, c.-422T>C, and c.-373G>A, and of two other SNPs, c.-302T>G and c.-628C>T, also located within the ANXA5 promoter.

Methods: A luciferase reporter assay was used to investigate the possible influence of the allelic variants on ANXA5 expression in HEK293 cells. Cells were transfected with constructs containing the ANXA5 promoter generated in the pGL3-Basic Vector. The allelic variants were generated using site-directed mutagenesis. Differences between wild-type and mutant ANXA5 luciferase activity were evaluated by one-way ANOVA. To identify DNA-protein complexes, EMSA was performed with nuclear extracts from BeWo cells and oligonucleotides carrying the various genotypes.

Results: Except for the c.-373G>A and c.-628C>T all other allelic variants affected the activity of the ANXA5 promoter as measured by luciferase activity. As assessed by EMSA, differential specific DNA-protein complexes were obtained for all allelic variants except for the c.-302T>G. This indicates that the examined polymorphisms in the ANXA5 promoter have an impact on the ANXA5 expression level.

Conclusion: We have functionally characterized allelic variants in the ANXA5 promoter, and found that several individual variants contribute to the modulation of transcriptional activity of the ANXA5 promoter, most likely through binding of nuclear factors. These results help to explain clinical findings and provide new insights into the mechanisms regulating the annexin A5 levels in healthy controls.

Disclosure of Interest: None declared.

PO454-TUE

Improvement in thrombin generation by the anti-TFPI antibody, concizumab, in plasma from individuals with haemophilia A with or without inhibitors or haemophilia B, is not affected by the presence of corn trypsin inhibitor

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Background: Inhibition of tissue factor pathway inhibitor (TFPI) may be an effective treatment for haemophilia by allowing sufficient thrombin to be generated by tissue factor (TF), factor VIIa, and factor Xa in the initiation phase of coagulation. Concizumab (mAb 2021) is a monoclonal IgG4 antibody targeting the Kunitz-2 domain of TFPI.

We showed, previously, that concizumab improves thrombin generation when added to plasma from patients with haemophilia in the absence of corn trypsin inhibitor (CTI). However there is much debate about the impact of CTI on thrombin generation.

Aims: To investigate the effect of CTI on thrombin generation in plasma from patients with haemophilia.

Methods: Blood was collected from 18 patients with haemophilia ($n = 9$ with severe haemophilia A, $n = 3$ with severe haemophilia B, and $n = 6$ with haemophilia A with inhibitors) in tubes with 3.2% trisodium citrate $\pm 50 \mu\text{g mL}^{-1}$ CTI. Platelet-poor plasma was generated from the blood samples and spiked with concizumab at concentrations from 0.001 to 500 nM. Thrombin generation was measured using 1 pM TF and 4 μM phospholipids.

Results: Concizumab improved thrombin generation when added to the plasma of all patients with haemophilia, regardless of diagnosis or inhibitor presence. Peak thrombin and endogenous thrombin potential were the most robust parameters. With >10 nM concizumab, these levels were nearly equivalent to control plasma from healthy individuals. The results were similar with both the inclusion and exclusion of CTI.

Conclusion: Concizumab successfully restored thrombin generation in plasma from patients with severe haemophilia A with or without inhibitors and haemophilia B. There was no discernible difference in thrombin generation when the blood was collected in either the presence or absence of CTI. These results indicate that in a controlled setting where all blood is handled in the same manner, CTI does not affect the improvement in thrombin generation by concizumab.

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PO455-TUE

Characterization of pro-coagulant antibodies binding to kunitz domain-1 of TFPI

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Background: Tissue Factor (TF) initiated coagulation is regulated through Tissue Factor Pathway Inhibitor (TFPI) mediated inhibition of Factor Xa (FXa) and TF/FVIIa. The TFPI Kunitz domain-1 (K1) inhibits TF-bound FVIIa and promotes the formation of a tight TFPI-FXa complex. Neutralizing TFPI inhibition prolongs TF/FVIIa-mediated activation of FX during initiation of coagulation and has the potential to promote haemostasis in haemophilia patients.

Aims: To determine the pro-coagulant effect of inhibitory anti-TFPI antibodies binding to the N-terminal region of TFPI (TFPI (1-79)), comprising the K1, under haemophilia-like conditions *in vitro* and address their mechanism of action.

Methods: Monoclonal antibodies (mAbs) were generated by standard hybridoma technology from mice immunized with human TFPI. Thrombin activity was assessed by calibrated automated thrombin generation measurements and thromboelastography. TFPI activity was assessed by chromogenic assays. Binding interactions were studied by Surface Plasmon Resonance analysis.

Results: Although the anti-TFPI (1-79) mAbs competed for binding to TFPI, they differentially affected thrombin generation and clot formation under haemophilia-like conditions. All antibodies bound with high affinity and neutralised TFPI inhibition of soluble TF (sTF)/FVIIa amidolytic activity. Whereas some mAbs were without an effect on FXa inhibition, the more potent pro-coagulant mAbs partially neutralised TFPI inhibition of FXa and were capable of dissociating a pre-formed TFPI/FXa complex.

Conclusion: We propose that the superior pro-coagulant activity of some of the anti-TFPI-K1 mAbs is due to a dual neutralising mechanism affecting TFPI inhibition of both sTF/FVIIa and FXa. In contrast, the less pro-coagulant active mAbs only affect TFPI inhibition of sTF/FVIIa.

Disclosure of Interest: H. Heibroch Petersen Employee of: Novo Nordisk, C. Augustsson Employee of: Novo Nordisk, B. Olsen Krogh Employee of: Novo Nordisk, J. Breinholt Employee of: Novo Nordisk, J. Thorn Clausen Employee of: Novo Nordisk, B. Kjør Employee of: Novo Nordisk, I. Hilden Employee of: Novo Nordisk, L. C. Petersen Employee of: Novo Nordisk.

PO456-TUE

The effect of ultra high levels of C-reactive protein (CRP) on the coagulation system

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Background: There has been conflicting evidence about the role of CRP in atherothrombosis as active mediator or innocent bystander in subjects with ischemic heart disease, stroke and peripheral arterial disease. Activation of inflammation and coagulations markers was previously demonstrated at intermediate in-vivo concentrations of Recombinant Human (rh) CRP.

Aims: The Purpose of this study was to evaluate the effect of ultra high physiologic levels of purified human (ph) CRP on coagulation system.

Methods: 300 microliter of Purified Human CRP concentrate synthesized from human pleural fluid (Lee Biosolutions, St. Louis, MO) was added to 1.8 ml test tube with citrated whole blood collected from 5 healthy male volunteers at escalating concentrations of 0, 10, 120, 250 ng L⁻¹ and was incubated for 30 minutes on a test tube rocker at room temperature. Coagulation parameters including, PT, PTT, D-dimer were analyzed through standard methods. Maximum Amplitude by Thrombelastograph TEG®5000 (Haemonetics, Braintree, MA) were measured with addition of calcium citrate to Kaolin assay. Measurements at different concentrations were compared to baseline values (without added CRP) using ANOVA test.

Results: There was no significant change noted in PT, PTT, D-dimer and the Maximum Amplitude by Thrombelastography with escalating doses CRP at physiologic ultra high levels.

Conclusion: CRP did not have a direct effect on the coagulation system in an isolated whole blood *ex vivo* coagulation model without contribution from the endothelial cells. The prothrombotic effect of CRP observed in previous studies may be attributed to the effect by the endothelial cells and furthermore likely by acute phase response due to contaminant bacterial products present in the production of bacterial based rhCRP used in contrast to the phCRP used in present study. The present findings suggest that elevated CRP observed in the clinical acute thrombosis is likely secondary inflammatory response rather than active contribution by the CRP.

Disclosure of Interest: None declared.

PO457-TUE

Assessment of the effect of inter-alpha trypsin inhibitor by thrombin generation assay

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Background: Inter-alpha trypsin inhibitor (ITI) is a circulating weak anti-protease implicated in several functions such as complement regu-

lation, matrix stabilization and anti-inflammation. The plasmatic ITI formed a family of proteins mainly constituted by 1 or 2 heavy chains (H1, H2 or H3) linked through a chondroitin sulfate to a light chain named, bikunin. Bikunin contains two kunitz protease inhibitor domains also present in the coagulation inhibitor Tissue Factor Pathway Inhibitor. A placental form of bikunin was showed to inhibit some of the coagulation proteases (Delaria et al., J Biol Chem. 1997).

Aims: Thus, we aimed to determine by using global assays whether plasma ITI may modulate coagulation.

Methods: ITI was purified from plasma at up to 96–98% of purity using three columns.

Results: ITI contained mostly H1 and H2 chains and no more than 10% in mass of H3 chains. The anti-protease activity of ITI was controlled on bovine trypsin and chymotrypsin, human trypsin, plasmin, elastase and cathepsin G. The data perfectly fitted with the results from the literature indicating that the molecule kept its anti-protease activity linked to the bikunin light chain. ITI was then added in normal plasma to reach 2- and 3-fold the normal circulating concentration. In these conditions the thrombin generation (TG) parameters (time to peak, peak height, ETP, lag time, velocity) were not significantly affected either when induced by a low level of tissue-factor (1 pM) or by cephalin. Normal plasma was 90% depleted on ITI by immuno-adsorption. A TG was evaluated on this plasma, and compared to the plasma treated with an irrelevant antibody. The signals were identical from the two matrixes. The TG assay was then performed in individual plasmas containing either a low or a high physiological level of ITI. No differences in thrombin generation can be evidenced whatever the level of plasma ITI.

Conclusion: As assessed by thrombin generation, the presence of ITI does not seem to modulate the coagulation.

Disclosure of Interest: J.-L. Plantier Employee of: LFB Biotechnologies, V. Devos Employee of: LFB Biotechnologies, S. Chtourou Employee of: LFB Biotechnologies, S. Jorieu Employee of: LFB Biotechnologies.

PO458-TUE

Prolongation of clot lysis time by a direct thrombin inhibitor: involvement of thrombin generation enhancement and thrombin-activatable fibrinolysis inhibitor

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Background: Previously we reported that a direct thrombin inhibitor melagatran paradoxically increased thrombin generation both *in vitro* and *in vivo*.

Aims: To test the hypothesis that melagatran may exert a deleterious effect on tissue-type plasminogen activator (t-PA)-induced fibrinolysis via enhancement of thrombin generation and activation of thrombin-activatable fibrinolysis inhibitor (TAFI), the effects of melagatran on clot lysis time were examined.

Methods: Pooled human plasma containing 100 ng mL⁻¹ t-PA and 10 nM thrombomodulin (TM) were mixed with anticoagulants and activated protein C (APC) or a TAFI inhibitor, potato tuber carboxypeptidase inhibitor. Clot formation was induced by 2.5 pM tissue factor and 4 µM phospholipids. To monitor the clot formation and lysis, the absorbance at 405 nm was measured every 30 s. Clot lysis time was defined as the interval between the time of the midpoint of the clear to maximum turbidity transition and the midpoint of the maximum turbidity to clear transition.

Results: In the presence of TM, melagatran at 300, 600, and 1200 nM significantly prolonged clot lysis time, but shortened it at higher concentrations. In the absence of TM, melagatran did not show any deleterious effects on fibrinolysis. Prolongation of clot lysis time by

melagatran was reversed by APC (which suppressed thrombin generation increased by melagatran) and a TAFIa inhibitor.

Conclusion: These results suggest that a direct thrombin inhibitor may induce clot formation that is resistant to fibrinolysis through the paradoxical enhancement of thrombin generation and subsequent TAFI activation.

Disclosure of Interest: Y. Morishima Employee of: Daiichi Sankyo Co., Ltd., C. Kamisato Employee of: Daiichi Sankyo Co., Ltd.

PO459-TUE

Oral factor Xa anticoagulants, rivaroxaban and apixaban, enhance clot dissolution in plasma

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Background: We showed that plasmin-cleaved clotting factor Xa (FXa) enhances clot dissolution by accelerating tissue plasminogen activator (tPA). Working toward improving this fibrinolytic function, a novel covalent active site modification of FXa resulted in further increased activity. The currently approved FXa-inhibiting anticoagulants, rivaroxaban (riva) and apixaban (apix) bind to the FXa active site in a similar manner as the covalent modification. A previously published study showed that riva may enhance plasma clot lysis by indirectly inhibiting thrombin generation and altering the clot structure. Here we address the hypothesis that these anticoagulants would further enhance the fibrinolytic function of FXa when *in situ* thrombin generation is carefully controlled.

Aims: 1) Investigate the effects of riva and apex on FXa cleavage. 2) Examine the tPA-cofactor function of FXa in the presence of riva and apex. 3) Determine the effects of riva and apex on clot lysis.

Methods: Thrombin-initiated clot lysis using normal and various factor-deficient plasmas or purified fibrinogen was followed by turbidity. The effects of riva and apex on the FXa-mediated enhancement of plasmin generation were examined by chromogenic assays (S-2251) in a purified system. FXa cleavage by plasmin was investigated by western blot, plasminogen ligand blot, and Coomassie blue protein staining.

Results: In plasma, riva and apex enhanced clot dissolution, but only in the presence of the FXa precursor, FX. This observation differed, however, in purified fibrin clot lysis as riva and apex inhibited the fibrinolytic function of FXa. Since *in situ* thrombin generation was carefully controlled, these results suggest the involvement of other plasma components or a different form of FXa in plasma compared to purified systems.

Conclusion: This study suggests a dual anticoagulant and profibrinolytic role for riva and apex and highlights their novel ability to enhance plasma clot dissolution.

Disclosure of Interest: R. Carter: None declared, K. Talbot: None declared, T. Smith: None declared, A. Lee Consultant for: Bayer and Bristol-Myers Squibb, E. Prydzial: None declared.

PO460-TUE

Ultrasonic monitoring of fibrin clot formation and fibrinolysis in flowing blood

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Background: The most dangerous thrombotic complications are associated with the formation of large thrombi in major arteries and veins of human organism. In such vessels intensity of blood flow profoundly influences the regulation of blood coagulation and fibrinolysis. In previous works we have demonstrated that ultrasonic methods are applicable for the monitoring of the early stages of thrombus formation under flow conditions. Here we present new experimental data on the ultrasonic monitoring of fibrinolysis.

Aims: The aim of our research was to analyze the kinetics of blood clots dissolution under flow conditions.

Methods: The research was performed on an experimental *in vitro* model of blood circulation. Data obtained by the ultrasonic monitoring of coagulation and fibrinolysis was analyzed in real-time. The infusion of fibrinolytic agents into the experimental system was governed by a computer in accordance with the data of ultrasonic monitoring. For this purpose a special drug-injector was designed. This experimental set-up enables us to infuse the drug in a precisely controlled manner during strictly determined stages of the blood coagulation. The efficiency of thrombi dissolution in the system was evaluated basing on quantitative ultrasonic parameters.

Results: It was shown that suggested ultrasonic technique can be used not only for monitoring of coagulation but also for quantitative analysis of fibrinolysis kinetics. Several protocols of infusion for various fibrinolytic agents (streptokinase, urokinase and tissue-type plasminogen activator) were tested and compared. It was established that under intensive flow conditions fibrinolytic resistance of blood clots increases greatly during the first 5 min of their formation.

Conclusion: Taking into account that the developed technique is principally non-invasive, it seems to be promising for the purposes of real-time clinical diagnostics.

The work was supported by Russian Science Foundation (RSF grant No.14-14-00990).

Disclosure of Interest: None declared.

PO461-TUE

A novel approach for hemophilia therapy through antibody targeted TFPI

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Background: The extrinsic coagulation pathway is one of the major physiological routes to thrombin generation. During the initiation phase of blood coagulation, tissue factor pathway inhibitor (TFPI), an endogenous inhibitor of the extrinsic coagulation pathway, blocks the coagulation signal.

Aims: Thus, we hypothesized that inhibition of the anticoagulant function of TFPI may allow appropriate clot formation. The objective of this study was to screen anti-TFPI antibodies which enhance blood coagulation.

Methods: For this, the mouse immune library has been screened to select anti-TFPI antibodies by using ELISA and SPR analysis and the lead antibodies have been further confirmed with several coagulation efficacy tests, including FXase and FVIIa/TF/FXa complex assay. At the last step, the hit antibody was humanized via anti-TFPI CDR grafting on a human IgG which was used as a template.

Results: We performed thrombin generation assay (TGA) and thromboelastography (TEG) with the humanized antibody to verify clot formation. In addition, we tested coagulation efficacy in an acquired hemophiliac model where the hemophiliac condition was induced by treating healthy donors with anti-FVIII neutralizing antibody.

Conclusion: Collectively, our results suggest that anti-TFPI antibody could be a potential therapeutic alternative for hemophilia patients.

Disclosure of Interest: None declared.

PO462-TUE

Functional components of Japanese traditional soybean food "NATTO"—nattokinase, vitamin K₂ and polyamine—

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Background: Nattokinase (NK) is a strong fibrinolytic enzyme and promote the release t-PA from the endothelial cells (Sumi *et al.*, *Experientia*, 43:1110, 1989; Yatagai *et al.*, *Pathophysiol. Haemost. Thromb.*, 36:227, 2009). Vitamin K₂ is important in bone metabolism with an essential factor in blood coagulation (Sumi, *Food Sci. Technol. Res.*, 5:48, 1999), and polyamine acts as a growth factor for various cells.

Aims: This study aimed to analyze and compare NK activity, vitamin K₂ and polyamine contents in natto products that received a prize at the "official National Awards Ceremony Recognizing Excellence (2010–2013)".

Methods: of the natto products entered in the above-mentioned competition, 49 types received prizes and were supplied by each company for our study. Determination methods were carried out as reported previously.

Results: All products showed strong fibrinolysis ($450 \pm 107 \text{ mm}^2/30 \mu\text{L}/4 \text{ h}$) and a strong specificity for substrate I (Bz-Ile-Glu-(OR)-Gly-Arg-pNA). In comparison, the specificity for the substrate II (Suc-Ala-Ala-Pro-Phe-pNA) decreased to less than half of that for substrate I. According to the substrate method, natto contains about 1.1 IU g^{-1} (wet wt.) of NK. Any activity that was neutralized with NK antibody has been estimated to be NK. Vitamin K₂ of these natto was higher ($896 \pm 364 \mu\text{g}/100 \text{ g}$) than the general natto. The main polyamine in natto was spermidine and spermine, at a very high concentration ($5.62 \pm 2.76 \text{ mg}/100 \text{ g}$).

Conclusion: A strong NK activity was confirmed in natto products obtained excellent results. And natto products proved to be superior in terms of vitamin K₂ and polyamine content. It is recognized that NK cannot be extracted from soy beans fermented using *Bacillus subtilis* rather than *Bacillus subtilis natto*. for example, there is no history of such products being used as food, leading to considerable misgivings about side effects and toxicity of natto produced using *Bacillus subtilis*.

Disclosure of Interest: None declared.

PO463-TUE

Fibrinolytic activity produced by fermented sweet corn with bacillus subtilis natto

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Background: In previous papers, we have presented reports on the physiochemical properties of nattokinase (NK), a constituent of natto (Sumi *et al.*, *Experientia*, 43:1110, 1987; Yatagai *et al.*, *Pathophysiol.*

Haemost. Thromb., 36:227, 2009). In addition, as a functional ingredient included in natto, osteoporosis with vitamin K₂ is now widely accepted.

Aims: We have researched an increase in production of the NK and other functions using substrates other than the soybean. In each case, the effect was insufficient compared to the soybean, except in the case of sweet corn.

Methods: We cultured Miyagino strain (4×10^3 pieces mL^{-1}) into sweet corn (Whole kernel type, MEIJI-YA Co., Ltd.) as *Bacillus subtilis natto* and cultured it in 37 °C, 48 h. Fibrinolytic activity, vitamin K₂, the following procedures were carried out as reported previously (Sumi *et al.*, *Food Sci. Technol. Res.*, 5:48, 1999). NK antibody and double immunodiffusion were carried out as reported previously (Sumi *et al.*, *ISTH*, PP-WE148, 2009; *New Food Industry*, 54:12, 2011).

Results: Sweet corn natto showed strong fibrinolytic activity. It showed thermal activity of 120 °C for 30 min. In contrast, normal corn natto had no activity. For each product tested, the fibrinolytic activity was completely neutralized with the antibody for NK. Borate saline buffer (BSB) at pH7.8 was used, and the NK showed strong activity for the substrate I (Bz-Ile-Glu-(OR)-Gly-Arg-pNA). Because activity declined to less than half the level of the substrate II (Suc-Ala-Ala-Pro-Phe-pNA) used for comparison, the true enzymatic activity of NK is believed to be at work. The measured value of vitamin K₂ content was 1890 $\mu\text{g}/100 \text{ g}$ on average, higher than general natto.

Conclusion: Sweet corn natto tested was shown through the lytic ability for the substrate I. It also contained a large amount of vitamin K₂. This fermentation technology was found to contain a larger amount of functional ingredients such as NK, vitamin K₂, which is expected to be used as a new food material in the future.

Disclosure of Interest: None declared.

PO464-TUE

Inhibition of fibrinolysis by heparin, low molecular weight heparin and a novel anticoagulant, antithrombin-heparin covalent complex (ATH)

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Background: Unfractionated heparin (UFH) and low molecular weight heparin (LMWH) are used to treat thrombosis. We have developed an antithrombin-heparin covalent complex (ATH) with faster rates of inhibition of coagulation factors vs. UFH and LMWH. Administration of ATH in a rabbit thrombosis model caused a decrease in clot mass, while clot mass increased with UFH treatment. Conversely, *in vitro* studies with purified proteins showed that ATH inhibits the rate of plasmin (Pn) generation significantly more than noncovalent UFH/AT mixtures. However, these *in vitro* studies did not account for the presence of other Pn inhibitors normally found in plasma, such as α_2 -macroglobulin (A2M).

Aims: To compare the effects of UFH, LMWH, and ATH on Pn generation in plasma.

Methods: Normal pooled plasma was supplemented with 0–2.1 U mL^{-1} of each anticoagulant and Pn generation initiated with tissue plasminogen activator and soluble fibrinogen fragments. At various times, subsamples were mixed with plasminogen activator inhibitor-1 to stop Pn generation and total chromogenically active Pn was quantified with S-2251. Some subsamples were also mixed with soybean trypsin inhibitor to inhibit free Pn, and remaining chromogenic activity due to Pn-A2M complexes (PAM) was quantified as above.

Results: Total generated Pn decreased slightly but significantly with 0.7 U mL^{-1} UFH or ATH, and 2.1 U mL^{-1} ATH. PAM generation decreased with 0.7 U mL^{-1} ATH. UFH and ATH had similar effects on total Pn and PAM generation at all doses. LMWH had no effect.

Conclusion: In plasma, ATH inhibited generation of Pn, but this effect was small and not significantly different from the effect of UFH. ATH may decrease PAM generation more than UFH. Future studies aim to determine the effect of ATH on Pn- α 2-antiplasmin generation and consumption of plasminogen. ATH's enhanced inhibition of coagulation factors combined with its limited effects on fibrinolysis may make it an effective treatment for thrombosis.

Disclosure of Interest: None declared.

PO465-TUE

Complement C3 and hypofibrinolysis: a potential role for protein glycation

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Background: Hypofibrinolysis is associated with increased vascular risk and complement C3 modulates the fibrinolytic process, particularly in diabetes. It remains unclear, however, whether C3 from diabetes patients has a differential effect on fibrin clot lysis compared with healthy control protein.

Aims: To investigate the potential differential effects of diabetic C3 on fibrinolysis, analyse inter-individual variability and elucidate underlying mechanisms.

Methods: Fibrinogen and complement C3 were purified from 8 patients with type 1 diabetes (T1DM), not on any treatment other than insulin, and 8 healthy controls. Fibrin clot lysis was determined using a turbidimetric assay and mass spectroscopy (MS) was used to analyse posttranslational modifications in the protein.

Results: Baseline characteristics were similar in T1DM patients and controls, except for HbA1c (78.4 ± 18 and 35 ± 4.1 mmol mol⁻¹, respectively; $P < 0.01$). C3 from T1DM showed a trend towards an exaggerated prolongation of clot lysis time compared with controls (800 ± 532 and 297 ± 148 s prolongation, respectively; $P = 0.076$). There was a large inter-individual variability with C3 prolongation in clot lysis in T1DM [44–1476 s (5–51%)] whereas in controls the range was much tighter [108–468 s (5–17%)]. MS ($n = 6$ in each group) showed increased glycation of C3 from T1DM patients in 5 separate Lys residues, which included K665, K1117, K1181, K1187 and K1505. More than one glycation site was noted in each of the samples studied with a mean of 3 lysine modifications (range 2–4).

Conclusion: Complement C3 from individuals with diabetes has a superior anti-fibrinolytic effect compared with protein purified from healthy controls. This may be due to increased protein glycation, potentially modulating C3-fibrinogen interactions. The large variability in the effects of C3 on fibrin clot lysis warrants further investigation with the possibility of C3- targeted individualized therapy to improve the hypofibrinolytic environment in diabetes.

Disclosure of Interest: None declared.

PO466-TUE

The arteriovenous difference of hemostasis parameters in critically ill patients with different type of hyperoergosis

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Background: The down-regulation of aerobic energy metabolism is a key pathogenetic link of any critical condition.

Aims: To reveal the regularity of hemostasis disturbances development in the venous and arterial vessels depending on type of hyperoergosis.

Methods: Were examined 199 patients underwent major abdominal surgery (gastrectomy, pancreatic-duodenal resection, hemicolectomy, liver resection, etc.). Patients was divided in five groups: the control group without hyperoergosis, and four groups with different types of hyperoergosis: substrate, hypermetabolic, hypoxic and enzymatic.

Results: For substrate hyperoergosis were characterized with minor changes in hemostasis, affects only coagulation; an arteriovenous difference were not differ from patients without hyperoergosis. For hypermetabolic hyperoergose most significant changes were observed in venous vessels – hypercoagulation, inhibition of fibrinolysis and elevated platelet aggregation. More significant comparison with the previous group changes in hemostasis determine formation of reliable arteriovenous difference of hemostasis, but direction of this difference was not differ from patients without hyperoergosis. For hypoxichyperoergosis were observed multidirectional disturbances of arteriovenous difference, with most prominent in arterial vessels – hypercoagulation, activation of fibrinolysis and increase of platelet aggregation. Consequently, were formed reliable arteriovenous difference of hemostasis with opposite direction to patients without hyperoergosis. For enzymatic hyperoergosis were insignificant arteriovenous difference of hemostasis, and have identical trend for arterial and venous blood: hypocoagulation, low platelets, decreased platelet aggregation, inhibition of fibrinolysis.

Conclusion: The trend and severity of founded metabolic disturbances, along with changes in arteriovenous difference, has statistically proven interrelation.

Disclosure of Interest: None declared.

PO467-TUE

Effects of an acidic environment on coagulation dynamics

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Background: Disruption of hydrogen ion homeostasis is a consequence of traumatic injury often associated with clinical coagulopathy. Mechanisms by which acidification of the blood contribute to the incidence of unexplained hemorrhage post-injury require further elucidation.

Aims: To examine the effects of acidified conditions on coagulation protein function in purified systems and whole blood.

Methods: A series of coagulation reactions were assessed at pH 7.4 and pH 7.0: 1) relipidated tissue factor (TF) (5 pM) added to the mixture of factors II, V, VII, VIIa, VIII, IX, X, and XI, TFPI and antithrombin (AT) $\pm$ (fibrinogen + FXIII) (all at mean physiologic concentrations), with reaction progress monitored as thrombin generation via chromogenic assay or fibrin formation via viscoelastic measurements; 2) thrombin cleavage of fibrinogen via viscoelastic measurements; 3) AT inhibition of FXa and IIa; 4) corn trypsin inhibitor treated phlebotomy blood $\pm$ 5 pM TF via viscoelastic measurements.

Results: The second order rate constants for AT inhibition of FXa and thrombin were reduced by ~25–30% at pH 7. Acidification induced relatively modest changes in TF-initiated thrombin generation: a ~15% increase in maximum thrombin levels, a ~10% increase in the time to maximum thrombin and a slower rate of thrombin clearance ($N = 3$). Parallel viscoelastic analyses showed a 25% increase in R time (clot time) and a 25% reduction in maximum clot strength (MCF) at pH 7. A similar reduction in MCF at pH 7 was observed when only FXIII and fibrinogen were reacted with thrombin. In contrast, in CTI blood ($N = 6$) at pH 7, R times decreased an average of 30% (range 12–43%) in TF initiated blood and an average of 50% (range 37–62%) in the absence of TF while MCF values ($\pm$ TF) were slightly elevated (~10%) in all donors.

Conclusion: Effects of acidification on coagulation dynamics in whole blood are not captured using acellular reaction systems constructed with purified components.

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PO468-TUE

Exercise-induced core body temperature elevations shift hemostasis to a prothrombotic state

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Background: Hemostatic parameters and core body temperature (CBT) can be influenced by exercise. This ultimate outcome is thrombosis for example observed in patients with heat stroke.

Aims: We assessed the relation between exercise-induced elevations in CBT and hemostatic changes.

Methods: CBT and hemostatic responses were measured in 62 participants of a 15-km road race at baseline and immediately after finishing. The individual CBT was measured with a calibrated ingestible temperature pill. Hemostatic responses were measured with the Nijmegen Hemostasis Assay, a global assay that measures thrombin and plasmin generation in plasma simultaneously. The assay temperature was synchronized to the individual's actual CBT at baseline and finish in a subgroup of $n = 25$.

Results: All subjects (44 ± 11 years, 69% male) completed the race at a speed of 12.1 ± 1.8 km h⁻¹. CBT increased significantly from 37.6 ± 0.4 °C to 39.4 ± 0.8 °C ($P < 0.001$). Post-exercise, hemostatic activity, expressed by accelerated thrombin generation and an attenuated plasmin response, was increased. The effect was more pronounced if the assay temperature was synchronized to the CBT. A similar temperature effect was also observed with normal pooled plasma. Hemostatic responses in subjects were comparable between low (<39 °C), moderate (39–40 °C) and high responders (≥ 40 °C) of finish CBT, though a significant interaction of CBT and fibrin lysis time was observed after correction of the assay temperature.

Conclusion: Exercise induces a prothrombotic state that is partially explained by exercise-induced rise in CBT. This study also shows that athletes with a finish CBT up to 41.2 °C do not demonstrate clinical symptoms of thrombosis. We hypothesize that a specific CBT threshold needs to be exceeded before derailment of the hemostatic balance. Finally, adjusting the assay temperature to the subject's CBT is recom-

mended to obtain essential insight in the hemostatic balance during thermoregulatory challenging situations.

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PO469-TUE

In vitro interactions of sugammadex with various anticoagulants

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Background: The selective muscle relaxant binding agent sugammadex (Bridion®) is associated with limited and transient increase in APTT. This modest increase does not translate into increased bleeding risks in patients treated with UFH/LMWH and sugammadex¹. We therefore explored *in vitro* the potential interactions on APTT of sugammadex with other common anticoagulants using UFH/LMWH as benchmark.

Aims: The aim was to investigate *in vitro* potential interaction between sugammadex and various anticoagulants.

Methods: Pooled plasma of healthy volunteers was spiked with UFH (0.15 U mL⁻¹), enoxaparin (5 and 10 µg mL⁻¹), fondaparinux (0.3 and 0.6 µg mL⁻¹), rivaroxaban (100 and 200 ng mL⁻¹) or dabigatran (106 and 212 ng mL⁻¹) covering the therapeutic ranges. Sugammadex was added at 50 and 200 µg mL⁻¹, corresponding with the plasma concentrations for clinical doses of 4 and 16 mg kg⁻¹. Readout measure was APTT.

Results: Sugammadex (50 µg mL⁻¹) prolonged APTT by an additional 5–6 s in the presence of the highest concentrations of UFH, enoxaparin, fondaparinux and dabigatran and by 9 s for rivaroxaban. A dose-dependent sugammadex-effect relationship was observed, independently of the anticoagulant concentration, with the exception of rivaroxaban, for which the relationship increased with increasing rivaroxaban (+7 and +18 s APTT at 100 ng mL⁻¹ rivaroxaban, and +9 and +30 s at 200 ng mL⁻¹ rivaroxaban, for 50 and 200 µg mL⁻¹ sugammadex, respectively).

Conclusion: Sugammadex prolonged APTT *in vitro* in the presence of UFH, enoxaparin, fondaparinux, dabigatran or rivaroxaban, with the strongest effect observed for the latter. The limited and similar prolongation in APTT observed for sugammadex with UFH/LMWH and sugammadex with fondaparinux/dabigatran suggest that also for the latter compounds a clinically relevant interaction *in vivo* may be unlikely, but the interaction with rivaroxaban may require further investigation.

¹ Anesthesiology, 2014 Nov;121(5):969–77.

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PO470-TUE

Coagulopathy and osmolar gap in critically ill patients: an intimate relationshipSukhanov V¹, Petrova O² and Levit A¹¹ICU, Regional Hospital #1; ²Regional Oncology Center, Ekaterinburg, Russia

Background: Endogenous intoxication (EI) (i.e., the difference between the measured and calculated osmolality – GAP) in critically ill patients (CIP) is often associated with coagulopathy.

Aims: The aim of our study was to evaluate the correlation between GAP and the severity of coagulopathy.

Methods: We studied 135 pts with multiple organ failure (M/F 71/64; mean age 47 ± 15 years) admitted in intensive care unit (ICU) independent of the cause of admission. Osmolality of blood was assessed with osmometer (Advanced Instruments™ Model 3320, USA). GAP – normal range ≤ 20 mOsm kg⁻¹. Investigated TEG (TEG analyzer 5000, USA) parameters were: R (our modification, RF patent #2015515) – time of reaction (normal range = 660–970 s), F (our modification, RF patent #2358657) – fibrinolysis (normal range = 60–120%). We evaluate the complex of hypercoagulant (HRP) and hypocoagulant (HOP) interaction of these two parameters. For this purpose we introduced a scoring assessment of their combined action: HRP–R < 500s = +1 score (normal range = 0), F < 40% = +1 score (normal range=0); HOP–r > 1500 = –1 score, F > 180% = –1 score. Severity of coagulopathy was determined by overall score (OS): high risk of thrombosis ≥ 2 scores, high risk of bleeding ≤ 2 scores.

Results: In 78 pts (58%) we found GAP ≥ 20 mOsm kg⁻¹. 62% pts had hyperfibrinolysis (F = –1 score, $r = 0.651$) and 35% – HOP ($r > 1500$ = –1 score, $r = 0.352$); OS = –1.4 ($r = 0.66$, $P < 0.05$). At the same time, 38% pts had hypofibrinolysis (F=+1 score; $r0.321$) and 43%–HRP ($r < 500$ = +1 score, $r = 0.552$), OS=+1.1 ($r = 0.48$, $P = 0.05$).

Conclusion: There is relationship between EI and severity of coagulopathy in CIP. Combined (score) assessment of R and F TEG parameters allows better estimate the clinical significance of EI.

Disclosure of Interest: None declared.

PO471-TUE

Net and neutrophil induced thrombin generationSong J¹, Lee E-Y², Park R³, Song Y-J⁴, Jo YA¹ and Yoo J⁵¹Laboratory Medicine, Yonsei University Health System, Seoul;²Laboratory Medicine, The Armed Forces Capital Hospital,Seongnam; ³Laboratory Medicine, Soonchunhyang UniversitySeoul Hospital; ⁴Yonsei University Health System, Seoul;⁵Laboratory Medicine, National Health Insurance Service Ilsan Hospital, Goyang, Korea

Background: Neutrophil extracellular trap is amorphous structure composed of chromatin strand complexed with nucleosome and other proteins released from stimulated neutrophils. The evidences of its role in the development of thrombotic disease are now mounting.

Aims: The direct role of NET in thrombin generation and coagulation factors contributing to NET assisted thrombin generation were examined.

Methods: Citrated plasma was added onto the NET containing medium and thrombin generation was measured by calibrated automated thrombogram with freshly isolated neutrophils as control.

Results: Spontaneous thrombin generation in the presence of NET always exceeded plasma alone or plasma with added PMA. The amount of thrombin was comparable between plasmas primed by NET and fresh neutrophils. The lag time of NET induced spontaneous thrombin generation was 9.8–15.7 s and 9.5–14.0 s when induced by viable neutrophils. The lag times for free plasma or plasma spiked with PMA were 19.1–29.1 and 18.9–28.2 s respectively. The peak height of

thrombin generation was 87–113 nM for NET and 87–129 nM for neutrophils, higher than free plasma (21–61 nM) or PMA plasma (20–46 nM). Endogenous thrombin potentials for NET and neutrophils were 585 1028 and 605–1113 and unmeasurable due to incompleteness for free plasma and PMA plasma. Thrombin generation was substantial when factor VII deficient plasma incubated with NET or neutrophils. Negligible amount of thrombin was generated in the absence of NET or neutrophil. Because NET induced factor XII activation has been demonstrated, we also examined thrombin generation in the factor XII deficient plasma with NET or neutrophils. Unexpectedly both NET and neutrophils induced significant thrombin generation. Substantial amount of thrombin was generated spontaneously in the presence of NET and neutrophils.

Conclusion: A complex underlying mechanism is responsible for NET or neutrophil induced coagulation activation.

Disclosure of Interest: None declared.

Reproductive issues II

PO472-TUE

Early anticoagulation and pregnancy outcomes in patients referred to a service of thrombosis and thrombophilia in pregnancy

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Background: Adverse pregnancy outcomes are common in patients with thrombophilia, mainly owing to placental insufficiency. The role of enoxaparin in the prevention of such complications is still unclear.

Aims: To evaluate the incidence of adverse pregnancy outcomes and thrombotic placental findings in patients with thrombophilia who received heparin during the first trimester and patients who started treatment after the first trimester.

Methods: Patients were referred to the service of Thrombosis and Thrombophilia due to a known thrombophilia, previous thrombosis or a bad obstetric past (previous stillbirth, preterm preeclampsia, recurrent fetal loss, placental abruption). We retrospectively selected 84 patients with precocious anticoagulation (group1) and 74 patients with anticoagulation after the first trimester (group 2) and analyzed the following outcomes in both groups: live term birth, intrauterine growth retardation, very low birth weight, fetal distress, intrauterine fetal death, placental abruption, eclampsia and thrombotic placental lesions.

Results: There was a higher incidence of previous VTE, the finding of any thrombophilia and a history of recurrent abortion in group 1. The group that did not receive heparin during the first trimester had a higher incidence of preterm birth. This group also showed a tendency toward an increased incidence of fetal distress, placental abruption and intrauterine fetal death, but this increase was not statistically significant. Concerning thrombotic placental lesions, no significant difference was observed between the groups.

Conclusion: These findings demonstrate that a probably poor trophoblastic invasion and placentation occurs in patients with thrombophilia who receive late anticoagulation, which could increase bad obstetric outcomes.

Disclosure of Interest: None declared.

PO473-TUE

Impact of hormone treatment in infertile women undergoing artificial reproductive techniques (ART) on thrombin generation, tissue factor, thrombomodulin and procoagulant phospholipid activities.

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Background: Blood hypercoagulability is implicated in the outcome of the artificial reproductive techniques (ART). However its role is not been sufficiently documented.

Aims: Infertile women eligible for ART were prospectively assessed for the impact of hormone treatment on thrombin generation (TG) and on the activities of Tissue Factor (TFa), thrombomodulin (TMa) and procoagulant phospholipid dependent clotting time (PPL) and their correlation with the ART outcome.

Methods: Eligible women for ART without any personal or family VTE history or known thrombophilia ($n = 38$) and 30 healthy matched women were studied. Blood samples were obtained at inclusion (T0), after hormone down-regulation (T1), FSH treatment (T2) and hCG injection (T3). TG was measured by CAT assay. TFa and TMa activities were determined by home made assays, and PPL by the STA-Procoag-PPL (DiagnosticaStago, France). The Upper Normal Limit (UNL) was determined in the control group as the mean + 2 SD.

Results: At T0 the ART group showed significantly increased TG vs. the control group. Associations of PPL/TFa or TMa/TFa higher to the UNL was found in 46% and 32% of ART negative women respectively and in 6% of ART positive women ($P < 0.05$). At T1 the associations of PPL/TFa or TMa/TFa higher to the UNL was found in 34% and 19% of ART negative women respectively and in 0% and 10% of ART positive women respectively ($P < 0.05$). At T2, TFa was higher than the UNL, in 56% in the negative ART, and 9% in positive ART. The association PPL/TFa was superior to the UNL in 25% of ART negative vs. 0% in ART positive ($P < 0.05$). At T3 the TFa and PPL were superior to UNL, in 36% and 28% of ART negative respectively, and in 0% in positive ART.

Conclusion: Steady state hypercoagulability is frequent in infertile women and is not amplified by treatment. Increased steady state levels of TFa, TMa and PPL are correlated with negative ART outcome. A larger study could allow to evaluate the clinical relevance of this association.

Disclosure of Interest: None declared.

PO474-TUE

New thrombophilic risk factors in patients with vascular placental complications (VPC) and pregnancy or hormonal therapy – related thrombosis and management in the clinical practice. Results from the international team project

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Background: Many questions regarding the management of CVG or thrombosis during the pregnancy and the possible association of thrombophilia with those complications as well as the need of thromboprophylaxis in those women are still unsolved.

Aims: We are conducting an international registry of women in these situations called TEAM project: a multicenter observational cohort study.

Methods: From 2009 almost 60 centres have participated. The Women TEAM's profile was: 1. Women with hormonal therapy or pregnancy-related thrombosis disease, 2. Women with history of VPC, 3. Thromboprophylaxis in women with VPC, 4. Thromboprophylaxis in women with thrombophilia. The study has been approved by the Ethical Committee of each Centre.

Results: Total of 587 episodes have been included, 77 belonged to profile one, 169 profile two, 138 profile three and 190 profile 4. In general, the most frequent thrombophilic factors were FII G20210A [rs1799963] (34 cases), factor V Leiden [rs6025] (62 cases) polymorphisms and new risk factors such as high factor VIIIc levels (30 cases). The most frequent factors in the VPC profile were the presence of anti-phospholipid antibodies (30 women), high factor VIIIc levels (9 women), deficiency of protein S (10 women), and also 12 women were carriers of the allele A1 of the genotype ABO and homozygous polymorphism F12 46C/T (4 cases).

We recorded termination of pregnancy and bleeding complications in treated or untreated with LMWH patients were performed. Low molecular weight heparin (LMWH) was prescribed in 75% of women and 25% was not. Minor bleeding was recorded in almost 2.4% episodes. No thrombosis or major bleeding related to treatment were observed.

Conclusion: The TEAM project reflect sthe differences among investigators in management of thrombophilic women during pregnancy. New thrombophilia factors seem to be important in this scenario such as Factor VIIIc and homozygous polymorphism F12 46C/T and the allele A1 (genotype ABO).

Disclosure of Interest: None declared.

PO475-TUE

Association between polymorphisms in Vascular Endothelial Growth Factor (VEGF) gene and repeated implantation failure (RIF)

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Background: The factors influencing a successful implantation are various including endometrium, thrombophilia, genetics, immunologic factors and so on. However, the etiology of repeated implantation failure remains a complicate challenge in terms of clinical approach. Successful implantation occurs at a short time between days 7 to 10 of secretory phase during normal menstrual cycle when the embryo develops to the blastocyst and migrates to the receptive uterus. The communication between the embryo and the uterus is crucial for synchronizing embryo development and uterine differentiation at the implantation window that are regulated by numerous pathways; hormones and signaling factors. In addition, vascular development or angiogenesis are very important factor for successful implantation.

Aims: To investigate the association of vascular endothelial growth factor (VEGF) polymorphisms (−2578C>A, −1154G>A, −634G>C, 936C>T) with repeated implantation failure (RIF) in Korean women.

Methods: Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses were performed for the −2578C>A and 936C>T genotypes. Real-time PCR (TaqMan allelic discrimination assays) was also used to analyze the −1154G>A and −634G>C genotypes. The 116 patients with a history of two or more unexplained consecutive implantation failure and 218 healthy controls with at least one live birth and no history of implantation failure.

Results: The GG (adjusted odds ratio [AOR] 2.543; 95% confidence interval [CI] 1.204–5.372) and CG+GG (AOR 2.090; 95% CI 1.048–4.165) genotypes of the VEGF −634G>C polymorphism was significantly different between women with RIF and controls. The differences in frequencies of the C-G-G-C, C-G-G-T, and A-A-G-C haplotypes of the VEGF polymorphisms (−2578C>A, −1154G>A, −634G>C, 936C>T) were marginally significant between the patient and control groups.

Conclusion: This study suggests that VEGF polymorphisms and haplotypes are a genetic determinant for the risk of RIF in Korean women.

Disclosure of Interest: None declared.

PO476-TUE

Maternal death and Venous Thromboembolism (VTE) in patients admitted in a maternity of high risk: results pre and post application of a risk score

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Background: Hospitalization in pregnancy and childbirth greatly increases the thromboembolic risk of these patients. The application of a protocol for assessing the risk of VTE reduces mortality and morbidity of these phenomena.

Aims: Report cases of venous thromboembolism (VTE) in hospitalized patients that occurred before and after the application of a protocol for prevention of VTE.

Methods: Descriptive, observational, prospective study. The data were collected between January 2013 and January 2015. A protocol for VTE prevention in all patients during hospitalization was applied since December 2013. Patients admitted to the service and who later developed venous thromboembolism were analyzed.

Results: There were 11 cases of venous thromboembolism during this period. The prevalence in 2013 corresponded to 2.95/1000 births, totaling 6 events in this period: 5 pulmonary embolism PE (4 PE and 1 PE plus DVT). There were two maternal deaths due to PE this year prior to the Protocol, one of which was associated with breast cancer and chemotherapy during pregnancy and the other with clinical chorioamnionitis and postmortem diagnosis of SC hemoglobinopathy and cor-pulmonale. After the systematic application of VTE prevention protocol, there were no more maternal deaths. There were two cases of PE, whose patients had very high risk score, but had bleeding risk due to placenta previa accreta. Another 3 DVT occurred respectively at 12th, 28th and 31th day postpartum and a superficial venous thrombosis (2th day postpartum) without identifiable risk factor. The risk assessment score failure rate was 0.33%.

Conclusion: The application of a risk score for VTE in hospitalized patients had a major impact on maternal mortality. Thromboembolic phenomena that occurred after the implementation of the protocol had no mortality and were minor. The failure rate assessment of the risk score was very low.

Disclosure of Interest: None declared.

PO477-TUE

A common genetic background shared by women with history of adverse pregnancy outcomes and cardiovascular disease: a window to future cardiovascular risk

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Background: A history of placenta-mediated pregnancy complications (PMPC) increases risk of cardiovascular disease later in life, possibly related to the persistence of endothelial dysfunction. In addition to classic cardiovascular biomarkers, there is a wide variety of 'non classic' cardiovascular markers associated with endothelial dysfunction, such as functional polymorphisms in candidate genes encoding for renin angiotensin system (RAS) and NO components which may represent a common genetic background shared by women with history of PMPC and vascular disorders

Aims: We investigated the prevalence of endothelium-related polymorphisms in 367 women with history of placenta-mediated pregnancy complications, compared with 290 women with history of premature cardiovascular events and 300 healthy women who delivered after uneventful pregnancy (HW).

Methods: All subjects were genotyped for 7 polymorphisms in ACE, AGTR1, AGT and eNOS genes

Results: ACE D allele frequency was similar between women with history of CVD and PMPC, and significantly higher than that observed in HW [OR (95% CI) 1.91, $P = 0.002$, and OR (95% CI) 2.18, $P < 0.0001$, respectively]. In women carrying ACE-240T or eNOS-786C allele a two-fold increase in stillbirth susceptibility was evidenced ($P = 0.004$, and $P = 0.005$, respectively). Women with history of stillbirth and premature CVD exhibited a significantly higher unfavorable allelic burden ≥ 3 in comparison to that observed in HW ($P < 0.0001$ and $P = 0.002$, respectively).

Conclusion: Our findings demonstrate a common genetic background shared by women with history of vascular disorders and placenta-mediated pregnancy complications; pregnancy may be considered a window to future cardiovascular risk, therefore 'non classic' genetic biomarkers of endothelial dysfunction might allow to identify women who could have a greater benefit for an early cardiovascular screening and prevention.

Disclosure of Interest: None declared.

PO478-TUE

Polymorphisms in tumor necrosis factor-alpha (-863C>A, -857C>T AND + 488G>A) are associated with idiopathic recurrent pregnancy loss in Korean women

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Background: Tumor necrosis factor-alpha (TNF- α) is a pro-inflammatory cytokine generated by lymphocytes and T cells that have been stimulated with antigen by mononuclear phagocytes as well as natural killer (NK) cells. Certain polymorphisms in the TNF- α gene affect cytokine production levels. Thus, cytokine gene polymorphisms have been hypothesized to play a role in idiopathic RPL. So we investigate the association between pro-inflammatory cytokine tumor necrosis factor- α (TNF- α) genetic polymorphisms and recurrent pregnancy loss (RPL).

Aims: The aim of this study was to investigate the association of three TNF- α polymorphisms, TNF- α -863C>A, TNF- α -857C>T and TNF- α + 488G>A. Blood samples were collected from 388 patients with idiopathic RPL and 224 control subjects

Methods: DNA was extracted from leukocytes using a G-DEX™ II Genomic DNA Extraction kit (Intron Biotechnology, Seongnam, Korea) according to the manufacturer's instructions. The TNF- α -863C>A, TNF- α -857C>T and TNF- α + 488G>A polymorphisms were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The patients were enrolled between March 1999 and February 2010 at the Department of Obstetrics and Gynecology of CHA Bundang Medical Center (Seongnam, South Korea).

Results: The TNF- α -863C>A variants increased risk of RPL (TNF- α -863CA + AA; adjusted odds ratio [AOR], 2.142; 95% confidence interval [CI], 1.493–3.074) and these data were not different in a stratified analysis according to the number of consecutive spontaneous abortions. Also, also in haplotype analysis, there were similar trends of data for combination analysis

Conclusion: In conclusion, this study showed significant associations between TNF- α -863C>A gene polymorphisms and RPL in Korean women. So, the results of these experiments, it is determined that TNF- α gene can have an effect on recurrent pregnancy loss in Korean women.

Disclosure of Interest: None declared.

PO479-TUE

The impact of mode of delivery and postpartum thromboprophylaxis on rotational thromboelastometry (ROTEM) in pregnant women with a high risk of postpartum venous thromboembolism

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Background: Pregnancy is a known hypercoagulable state with venous thromboembolism a leading cause of maternal morbidity and mortality. Anticoagulant and mechanical thromboprophylaxis (TP) for women at high-risk postpartum is therefore recommended, although optimal risk stratification remains uncertain. ROTEM has previously demonstrated hypercoagulability in normal pregnancy.

Aims: To investigate the impact of mode of delivery and postpartum TP on hypercoagulability as measured by ROTEM.

Methods: Ethical approval and consent were obtained. Women likely to require postnatal TP according to local guidelines were recruited. Bloods were taken pre delivery (T1), post delivery (T2), post 1st dose prophylactic enoxaparin (T3), and at 6 weeks (T4) for ROTEM. Parameters of interest were INTEM clotting time (CT), clot formation time (CFT) and maximum clot firmness (MCF) and FIBTEM MCF (FMCF).

Results: One hundred and one women were recruited with mean age 35.8, BMI 29, parity > 2 in 35.7% of cases. Vaginal deliveries (VD) accounted for 32.7% of deliveries. Median number of postpartum risk factors in VD was 2 and caesarean section (CS) 3. At T1 and T2 ROTEM profiles were hypercoagulable with shortening of CT, CFT with increased MCF & FMCF compared to T4. At T3 these changes were attenuated. At T2 post VD, there was significantly shorter CFT (46.5 vs. 54, $P = 0.002$), and higher MCF (73 vs. 71, $P = 0.001$) and FMCF (28 vs. 23, $P < 0.001$) compared to CS. At T3, post enoxaparin, both groups showed shortened CT and CFT, more pronounced post VD (CT 148 vs. 159, $P = 0.035$; CFT 47 vs. 50, $P = 0.026$). At T4 there was no significant difference between groups, with normalisation to presumed pre-pregnancy results.

Conclusion: ROTEM illustrates hypercoagulability associated with pregnancy and delivery; this is attenuated by thromboprophylaxis and resolved at 6 weeks. The ROTEM changes at T2 demonstrate enhanced hypercoagulability in those post VD compared to CS, suggesting current UK risk stratification and recommendations for postpartum thromboprophylaxis are appropriate.

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PO480-TUE

Retrospective study of patients who were treated with fondaparinux pre-, peri- and/or postpartum for prophylaxis or treatment of venous thromboembolism (FONDAPPP)

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Background: Low-molecular weight heparins (LMWH) are the preferred agents for anticoagulation in pregnancy. Still, in a considerable proportion of pregnancies heparin intolerance (allergic reactions, HIT, increases in liver enzymes or other events) make it necessary to change to another anticoagulant. Fondaparinux is a synthetic selective indirect inhibitor of factor Xa with a favourable efficacy/safety profile compared to LMWH.

Aims: In this retrospective multi-center study we descriptively analyzed data of women who had been treated for ≥ 7 days with fondaparinux during pregnancy.

Methods: In total, 120 women (mean [$\pm$ SD] age 31.5 ± 5.4 years) from 7 specialist centres were included. Of 85 women with former pregnancy 60.0% had suffered ≥ 1 abortion. The indication of anticoagulation was prophylactic in 92.5% of all women, in 99 women (82.5%) specifically due to an elevated VTE risk. Of these, 82.8% had

known thrombophilia (mostly without previous VTE) and 33.3% a history of VTE. All women received LMWH first-line (median treatment duration: 54 days) (3 additionally received UFH). Mainly due to heparin allergy or heparin-induced thrombocytopenia (HIT-2) treatment was changed to fondaparinux (predominant dose 2.5 mg day⁻¹; median treatment duration: 131 days).

Results: Fondaparinux was well tolerated, with no complications reported in 111 of 120 patients. Adverse events included one case of abdominal wall hematoma, four cases of vaginal hemorrhage, one hemorrhage of other location, one drop in platelet count without thrombotic complications, one abortion, one premature birth, one stillbirth, and one child born with trisomy 18. Thromboembolic events, allergic reactions, or increased liver enzymes, did not occur during fondaparinux treatment.

Conclusion: In this retrospective study, a notable number of pregnant women at risk of VTE and with intolerance to LMWH received successful and safe long-term treatment with fondaparinux.

Disclosure of Interest: C.-E. Dempfle Speaker Bureau of: Aspen Europe GmbH, J. Koscielny: None Declared, E. Lindhoff-Last: None Declared, J. Oldenburg: None Declared, H. Pollmann: None Declared, G. Kappert: None Declared, U. Scholz: None Declared, S. Kropff Employee of: Glaxo-Smith-Kline GmbH, S. Eberle Employee of: Winkler-Norimed GmbH, A. Heinken Employee of: Aspen Europe GmbH.

PO481-TUE

Rotational thromboelastometry in pregnancy and its role in assessment of haemostasis in women with factor XI deficiency

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Background: Women with FXI deficiency are at an increased risk of bleeding complications at delivery. Obstetric management is complicated by a lack of correlation between FXI level and bleeding risk.

Aims: This study had two aims; firstly to assess the difference in rotational thromboelastometry (ROTEM®) in pregnant women with FXI deficiency compared to parturient and non-parturient controls; and secondly to evaluate the usefulness of ROTEM® in assessing bleeding risk at delivery in women with FXI deficiency.

Methods: ROTEM® was performed on 60 women; 27 with FXI deficiency, 20 age-matched parturient controls during the third trimester of pregnancy, and 12 non-parturient controls. We reviewed ROTEM® analysis, pregnancy outcomes and haemostatic cover in 57 deliveries of women with FXI deficiency. Women with FXI deficiency were categorised according to how ROTEM® parameters were in keeping with normal pregnancy

Results: Women with FXI deficiency had longer clotting time (CT) compared to parturients ($P = < 0.001$), but shorter clot formation time (CFT) ($P = < 0.001$) and increased alpha angle ($P = < 0.001$) compared to non-parturients. The maximum clot strength was increased in FXI deficiency patients compared to non-parturients ($P = 0.005$) and no difference in clot lysis was detected between the groups. Postpartum haemorrhage occurred in 11/57 (19.3%) of deliveries. In each case there were additional obstetric risk factors.

Conclusion: ROTEM® is useful in assessing the overall haemostatic function in women with FXI deficiency in pregnancy. An individualised risk assessment incorporating the bleeding history, FXI levels and ROTEM® is necessary for deciding appropriate haemostatic cover at delivery.

Disclosure of Interest: None declared.

PO482-TUE

Thrombin generation in women undergoing ivf treatment is prolonged, but not enhanced compared to controls

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Background: It has been described, that during *in vivo* fertilization (IVF) treatment a procoagulant state, expressed by increased thrombin generation (TG), is reached; the implication of this finding are unclear.

Aims: The aim of the study was to evaluate the changes in the coagulation potential during IVF treatment by measuring thrombin generation and to compare the results to those obtained from healthy women not taking any hormonal treatment during the menstrual cycle.

Methods: The patient cohort consisted of 26 women undergoing IVF. Blood samples were collected during: (i) the down-regulation phase, (ii) 5–8 days prior to oocyte retrieval, (iii) 2–3 days prior to oocyte retrieval and (iv) during oocyte retrieval.

Blood samples from 102 healthy women were collected during the follicular and the luteal phase of a menstrual cycle and were used as controls (mean values). TG was measured by the Calibrated Automated Thrombogram (CAT®) in all samples. The markers measured were lagtime, endogenous thrombin potential (ETP), peak thrombin concentration and time to peak (tpeak).

Results: ETP and peak were significantly lower during phase 1 compared to the three other phases whereas peak was significantly higher during phase 4 compared to phases 2 and 3. On the contrary, tpeak was significantly higher during phase 1 compared to phases 2, 3 and 4. When thrombin generation in patients was compared to the values obtained from controls (mean values), it was shown that ETP was slightly lower during phase 1 compared to controls, but otherwise no significant differences were observed. The 'time' parameters (lagtime and tpeak) as well as the peak thrombin generation were significantly prolonged during IVF (all phases) compared to controls.

Conclusion: It appears that TG is prolonged, rather than enhanced during IVF treatment compared to controls. Whether this prolongation is linked to increased procoagulant activity and risk for thrombosis is unclear and more studies are required.

Disclosure of Interest: None declared.

PO483-TUE

Low-molecular –weight heparin in pregnancies after art: a randomized study

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Background: The number of embryo implantation in Assisted Reproductive Techniques (ART) remains low, despite new techniques or the off-label use of antithrombotic drugs, as heparins or aspirin. The potential role of thrombophilia in worsening the implantation rate is still debated.

Aims: The aim of this first prospective and randomized trial was to evaluate the potential benefit in pregnancy rate of parnaparin, used at body weight adjusted dose (4250 UI if body weight under 60 kg, 6400 UI if above 60 kg), once per day subcutaneously, starting from ovulation induction until delivery in ART.

Methods: We enrolled 256 infertile women (mean age 35.8, 26–40 years old; idiopathic infertility 9.38%; primary infertility 66.2%; first ART attempt: 35.94%, ≥three ART attempts: 18%), undergoing a new

ART (91.3% ICSI, 8.30% FIVET), prospectively randomized to receive or not studied treatment. We excluded women with severe thrombophilia (antiphospholipid antibodies, FVLeiden or FIIG20210A omozygous, FVLeiden+FIIG20210A eterozygous, natural anticoagulants deficiency) or previous thrombosis. Testicular or frozen sperms, aspirin or steroids were not allowed. Participants gave written informed consent, and were shared in three age groups: ≤ 35 (38.28%), 36–38 (26.41), 39–40 (37.11%).

Results: Total thrombophilia's carriers were 11.3%. We obtained 33 (25.58%) pregnancies in not-treated and 27 (21.26%) in treated group (P 0.462). No significant differences also analyzing the three age groups: ≤ 35 (17 vs. 12%), 36–38 (18 vs. 20%), 39–40 (22.58 vs. 15.63). The study is ongoing and definitive data about life-born children are not available, but preliminary results document a lower miscarriages rate in treated group (2.36 vs. 4.65).

Conclusion: In this first prospective randomized trial we don't document efficacy of parnaparin to avoid implantation failure in ART, despite of thrombophilia, but it could be possible to demonstrate a positive effect on life-born children rate. We confirm mild thrombophilia doesn't influence pregnancy outcome in ART.

Disclosure of Interest: None declared.

PO484-TUE

Effects of sildenafil citrate on coagulation process during pregnancy: a promising therapeutic approach in combination with low molecular weight heparin for recurrent pregnancy loss

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Background: Acute and recurrent pregnancy losses (RPL) are common women's health issues occurring in 20% of the pregnancies. Indeed inflammatory and thrombotic events have been associated with spontaneous pregnancy loss. New approaches are needed to improve the treatments of women suffering with RPL. Sildenafil Citrate (Sil); a phosphodiesterase-5 inhibitor is an FDA authorised drug to use for erectile dysfunction and pulmonary hypertension. However was also used for treatment of fetal growth restriction. The effect of Sil on coagulation and the understanding of the mechanism involved in RPL in relationship to coagulopathies would be essential for optimal treatment modalities.

Aims: To investigate the effects of Sildenafil on maternal blood coagulation and fetal health in association with recurrent pregnancy loss

Methods: RPL model was induced in pregnant Albino Swiss mice by injection of lipopolysaccharides (LPS) ($100 \mu\text{g kg}^{-1}$) at gestational day (gd) 15.0. Mice were treated with Sil (50 mg kg^{-1}) and Low Molecular Weight Heparin (LMWH) (500 IU kg^{-1}) starting at gd 0, either separately or in combination, and were compared to saline injected control mice. Mice blood samples were collected and used for Thrombelastography (TEG) analysis and the fetuses were clinically analysed. Healthy women's blood samples were treated ex vivo with Sil (200 ng mL^{-1}) and LMWH (0.2 IU mL^{-1}) and used for TEG analysis. Two way ANOVA was used for statistical analysis

Results: Combined Sil and LMWH treatment improved the health status of the fetuses, improved the LPS induced coagulopathy and brought TEG parameters close to the control group even after LPS administration. Sil improved significantly MA, LY30 and CI in mice and Sil alone did not change the coagulation pattern in normal women's blood samples but potentiated the LMWH effects

Conclusion: Sil is a safe drug and represents a potential novel therapeutic target to RPL, especially as an adjuvant to LMWH treatment

Disclosure of Interest: None declared.

Risk Factors Venous Thrombosis II

PO485-TUE

A risk score for prediction of recurrence in patients with unprovoked venous thromboembolism (damoves)

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Background: In patients with unprovoked venous thromboembolism (VTE), the optimal duration of anticoagulation is anchored on estimating the risk of disease recurrence.

Aims: We sought to develop a simple risk score for prediction of the recurrence risk in patients with a first unprovoked VTE.

Methods: Patients with a first unprovoked VTE were recruited from Hospital de Torrejón and Hospital de Fuenlabrada, between March 2004 and August 2013. They were treated with oral anticoagulants for at least 3 months. We excluded patients with a strong thrombophilic defect. Preselected clinical and laboratory variables were analyzed based of the independent confirmation of the impact on the recurrence risk, simplicity of assessment, and reproducibility. We developed a nomogram to calculate risk score of recurrence in an individual patient.

Results: In a prospective cohort study 398 patients with a first unprovoked VTE were followed up for a median of 21, 3 months after discontinuation of anticoagulation. A total of 65 patients (16.3%) had recurrent VTE. In all patients, VTE recurred spontaneously. Male sex (HR=2.89 [95% CI 1.21–6.90] P = 0.016), age (HR=1.03¹⁰ per additional decade [95% CI 1.01–1.07] P = 0.011), obesity (HR=3.92 [95% CI 1.75–8.75] P = 0.0001), varicose veins (HR=4.14 [95% CI 1.81–9.43] P = 0.0001), abnormal D-dimer during anticoagulation (HR=13.66 [95% CI 4.74–39.37] P = 0.0001), high factor VIII coagulant activity (HR=1.01 [95% CI 1.00–1.02] P = 0.028) and heterozygous of factor V Leiden and/or Prothrombin G20210A mutation (HR=13.86 [95% CI 5.87–32.75] P = 0.0001) were related to a higher recurrence risk. According to the nomogram, a score < 11.5 points was considered a low recurrence risk.

Conclusion: The DAMOVES prediction model may be useful to decide whether anticoagulant therapy should be continued indefinitely or stopped after an initial treatment period of at least 3 months in patients with a first unprovoked VTE. The model should undergo external validation before it is applied in routine clinical practice.

Disclosure of Interest: None declared.

PO486-TUE

Analysis of prothrombin mutants in NA+ binding domain as a potential candidate conveying antithrombin resistance

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Background: Antithrombin (AT) resistance (ATR) is a risk of thrombosis, because the mutant thrombin is impaired in inactivation by AT resulting in a prolonged procoagulant activity. We have reported 2 ATR mutations in prothrombin gene (p.R596L, prothrombin Yukuhashi; p.R596Q, prothrombin Belgrade). On the basis of protein structure prediction in thrombin-AT complex, 596R lies in the Na⁺ binding

region and forms hydrogen bonds directly between 265N of AT. 599K also binds by a hydrogen bond directly to 264E of AT, and 540T, 541R and 592E bind indirectly to AT. 509E in thrombin forms intramolecular hydrogen bonds between 596R.

Aims: We aimed to evaluate ATR of each mutant prothrombin as a possible candidate for the risk of thrombosis.

Methods: Expression vectors of wild-type and 30 distinct mutants caused by single nucleotide substitution at 599K, 540T, 541R, 592E and 509E were constructed. After stable transformants of HEK293 cells were established, we prepared respective recombinant prothrombins. We measured procoagulant activity of each recombinant by two-stage chromogenic assay using S-2238. To evaluate ATR, we prepared each reconstituted plasma by mixing prothrombin deficient plasma and recombinant prothrombin, and measured the residual thrombin activity during inactivation by AT using modified our ATR detecting assay. The effect of each mutation on the protein structure prediction in thrombin-AT complex was predicted.

Results: All recombinant prothrombins were applicably secreted. Procoagulant activity of each mutant varied from 107.8% to 2.1% in two-stage chromogenic assay. In addition to 2 reported ATR mutations (596L and Q), all 599 and 540 mutants, as well as 592 mutants except for 592V, showed severe ATR. All 541 mutants and 592V showed relatively mild ATR compared with the other mutants. It was forecasted by *in silico* analysis that each mutation disrupted conformation of the molecule and hydrogen bounds.

Conclusion: Some of prothrombin mutations tested may have a potential risk for thrombosis due to ATR.

Disclosure of Interest: None declared.

PO487-TUE

Oral anticoagulant therapy for the prevention of recurrent thrombosis in patients with polycythemia vera or essential thrombocythemia

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Background: A third of patients with polycythemia vera (PV) or essential thrombocythemia (ET) experience a thrombotic episode at disease diagnosis or during follow-up. However, it is unclear whether the clinical management of anticoagulation intended for the general population is applicable to this particular subset of patients.

Aims: To analyze the risk of thrombotic recurrence in a multicenter series of PV and ET patients who had an arterial or venous episode at disease presentation or during follow-up and received oral anticoagulation therapy with vitamin K antagonists (VKAs).

Methods: We have retrospectively compared the incidence of recurrent major thrombosis and hemorrhage during the observation periods in which the patients were with or without VKA therapy, and tried to identify the risk factors associated with these vascular complications.

Results: A total of 150 patients (79 PV, 71 ET) had at least one thrombotic episode, mainly involved the venous territory (116 or 77% cases). After an observation period of 963 patient-years, the incidence of recurrent thrombosis was 6.0 per 100 patient-years. History of thrombosis prior to the diagnosis was a predictor of recurrent venous thrombosis (IRR: 9.8, 95% CI: 3.3–29, $P < 0.001$), while cardiovascular risk factors (IRR: 7.0, 95% CI: 1.8–26, $P = 0.004$) and the *JAK2* mutation (IRR: 10.0, 95% CI: 1.5–80, $P = 0.03$) increased the risk of arterial recurrences. The recurrences were significantly lower while the patients were on VKA (4.5 vs. 12.0 per 100 patient-years, $P < 0.0005$). VKA treatment was associated with a 2.8-fold reduction in the risk of thrombotic recurrence. There was no significant difference in the incidence of hemorrhage according to whether the patient was or not on VKA treatment (1.8 vs. 1.5 per 100 patient-years, respectively, $P = 0.8$).

Conclusion: Our results support the use of long-term VKA therapy for the prevention of recurrent thrombosis in patients with PV or ET.

Disclosure of Interest: None declared.

PO488-TUE

ACSF2 expression and risk of recurrence after a first unprovoked deep venous thrombosis event. Results from the Retro Study

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Background: Provided the bleeding rates associated with anticoagulant therapy, the identification of patients with unprovoked venous thromboembolism with higher recurrence risk would improve the selection of those who would benefit most from extended therapy.

Aims: To assess differences in the gene expression profile of peripheral blood cells between patients with or without recurrence after withdrawal of anticoagulant therapy for a first episode of unprovoked deep vein thrombosis (uDVT), in order to identify novel predictors of recurrence.

Methods: Patients with and without recurrent thrombosis after a first uDVT event were recruited. In the discovery population ($N = 32$), a microarray RNA assay was performed. In the validation population ($N = 44$) a multiple RT-PCR-based strategy was applied to assess those genes differentially expressed in the discovery population.

Results: In the discovery population the sex-adjusted Linear Model for Microarray Data (LIMMA) analysis showed 102 genes differentially expressed ($P < 0.01$) between patients with or without recurrent DVT. Following biological plausibility criteria, 19 of them underwent further confirmation in the validation population. The gene encoding for Acyl-CoA Synthetase Family Member 2 (*ACSF2*) was underexpressed in recurrent DVT patients in both, the discovery ($P = 0.007$) and validation population ($P = 0.004$). In the receiver operator characteristic

(ROC) analysis, ACSF2 expression showed sensitivity and specificity over 70%.

Conclusion: We have identified for the first time an association between ACSF2 expression and the risk of recurrent DVT. Should this association be confirmed in larger prospective studies, ACSF2 would become an attractive predictor to help in the selection of patients requiring extended anticoagulant therapy.

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Disclosure of Interest: None declared.

PO489-TUE

Prospective observational study on incidence of venous thromboembolism in medically-ill hospitalized elderly patients

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Background: Pharmacologic thromboprophylaxis (TP) is recommended for hospitalized medical patients with risk of thrombosis. Advanced age is a risk factor for venous thromboembolism (VTE) and elderly compose the most of medical in-patients. Ethnic differences in incidence of VTE in hospitalized medical patients seem to exist between Korean and western people.

Aims: We conducted a prospective observational study to estimate the incidence of asymptomatic VTE in medically-ill hospitalized elderly patients.

Methods: Medical patients who were older than 65 years, whose projected stay in the hospital at least 5 days were eligible. Patients who had known thrombophilia, a life expectancy of < 3 months, and took anticoagulation were excluded. Patients were examined for deep vein thrombosis (DVT) by duplex and color Doppler ultrasonography (DUS) of both legs.

Results: Between Feb 2012 and July 2014, 201 patients were screened with DUS and 185 eligible patients were analyzed. The median age was 73 years (range 65–95) and 54% were female. The median duration of hospitalization was 13 days and 73% of the patients had active malignancy including 118 solid and 36 hematologic malignancies. Padua prediction score ≥ 4 was 82.7%. The incidence of DVT detected by DUS during hospital stay was 8.6% ($n = 16$) including 9 proximal DVT, and all cases were asymptomatic. The incidence of DVT in patients with solid or hematologic malignancies and other benign illnesses were 9.3%, 8.3% and 6.5%, respectively ($P = 0.877$). Nine of 16 patients received anticoagulation treatment (ACT) and 3 completed planned ACT. Cases of early stopping ACT were caused by bleeding complication ($n = 2$), deconditioning related with infection and uncontrolled underlying malignancy ($n = 2$) and death of unknown cause ($n = 1$).

Conclusion: Korean elderly hospitalized patients had comparable risk of DVT when compared with that of western population, and pharmacologic TP for high-risk group should be considered.

Disclosure of Interest: None declared.

PO490-TUE

Inherited prothrombotic risk factors in children with intracranial venous thrombosis: single center experience in Turkey

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Background: Intracranial venous thrombosis (IVT) is a rare condition in childhood, with a wide variety of clinical features and etiologies.

Aims: We aimed to describe the inherited prothrombotic risk factors in children with IVT, confirmed by neuroimaging.

Methods: The retrospective study involved reviewing the records of patients who were admitted to our hospital during the years of 2010–2014. The patients diagnosed as having IVT, confirmed by neuroimaging were investigated for the common thrombophilia markers, such as protein C (PC), protein S (PS), antithrombin III (AT III), factor V G1691A and prothrombin 620210A mutations, methylenetetrahydrofolate reductase (MTHFR) C677T, and MTHFR A1298C genotypes.

Results: The clinical manifestations in 18 patients with IVT included headache, seizures, cranial nerve palsy, and hemiparesis. Transfer sinus thrombosis was the commonest site (50%) followed by diffuse sinus thrombosis (31.25%). Two patients had PC deficiency. Furthermore, one of them had homozygous MTHFR C677T genotype. No patient had PS and ATIII deficiencies, and prothrombin 620210A mutations. Eleven patients showed the MTHFR genotype (homozygous C677T, $n = 4$, heterozygous C677T, $n = 5$, heterozygous A1298C, $n = 2$); and 1 patient was carrier of heterozygous factor V mutation.

Conclusion: Early diagnosis by fast and safe radiological methods (neuroimaging of the brain), investigation of thrombophilia markers and the appropriate anti-clotting therapy in acute phase may prevent death due to IVT at the pediatric age.

Disclosure of Interest: None declared.

PO491-TUE

Thromboprophylaxis skipped doses in hospitalized patients. Can we recognize which are the causes or factors?

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Background: We know that a large number of hospitalized patients are at risk for Venous Thromboembolic (VTE). In order to reduce patient's VTE risk in our Institution, we wrote our own thromboprophylaxis guidelines based on 2008 and 2012 American College of Chest Physicians (ACCP) guidelines. We also measured medical staff guideline adherence every 6 months. Nowadays we know that we have a 87% guideline adherence in medical and surgical patients. Having already improved this, clarifying which are the causes for skipping doses, this will be our next step to focus in order to improve thromboprophylaxis adherence.

Aims: Establish the number of skipped doses per patient and the association between skipped doses and some analyzed variables

Methods: Retrospective, analytical and descriptive study of 104 hospitalized patients. Variables analyzed: age, gender, length of stay, programmed vs. urgent admission, change of hospitalization area, skipped doses (interruption of prophylactic doses that have been justified or not) and modifications of doses. A logistic regression analysis was performed to identify causes factors that were associated to skipped doses.

Results: Fifty-five per cent patients without skipped doses, and 1 skipped dose every 11.80 days. Average days of hospitalized 12.90 days. In general, we observed more skipped doses in patients with a larger length of stay, patients that are at urgency for admission, patients that change of hospitalized area. After all adjusted factors, the interruption of dose (justified or not) is an independent factor from skipped doses with an odds ratio 12.53 (IC 4.48–34.98)

Conclusion: To improve our work we need to detect strengths and weaknesses. When patients need to interrupt prophylaxis for different circumstances as invasive procedures, the prophylaxis enters in the more dangerous area for skipped doses followed by changes in hospitalization area and in length of stay. All this information will help us to be extremely careful when the patient is placed in any of these circumstances.

Disclosure of Interest: None declared.

PO492-TUE

Identification of a novel homozygous mutation responsible for type II (P.S169F) antithrombin deficiency

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Background: Antithrombin deficiency increases the risk of thrombosis. Complete quantitative deficiency results in intrauterine lethality.

Aims: Here we report a patient who is viable with homozygous antithrombin deficiency.

Methods: The proband is a 37-year-old male who developed a deepvein thrombosis. He underwent a serial of thrombophilic tests including antithrombin activity, protein C activity, protein S activity, and anti-phospholipid antibodies. The antithrombin antigen was measured, when a declined antithrombin activity was observed. Then a mutation analysis was performed by sequencing the whole *SERPINC1* gene comprising promoter region, seven exons and the flanking intronic regions. To evaluate the influences of the mutation on blood coagulation, thrombin-generation tests (TGT) were performed.

Results: A C5462T (p.S169F) mutation in the exon3 of antithrombin gene was identified. The patient showed mildly reduced antithrombin activity, while other tests for the detection of thrombophilia were normal. Thus, the anti-IIa activity in the presence of unfractionated heparin was 31% (reference range, 80% > 120%), whereas the anti-IIa activity in the presence of low molecular weight heparin was 49% (reference range, 80% > 120%). Nevertheless antithrombin antigen concentration was within the normal range. Higher TGT parameter with an increased endogenous thrombin potential value (1340.21 nm min⁻¹, reference range, < 835 nm min⁻¹) was observed.

Conclusion: This is the first report of a novel homozygous type II antithrombin mutation in China. Mutations in the heparin-binding site of antithrombin have been believed to be of low importance with regard to thrombosis risk. Accordingly, further studies must be performed to clarify the effect of this mutation causing heparin-binding defects. Our results indicate that the use of heparin in these patients may be ineffective as the mutation impairs the activation induced by heparin.

Disclosure of Interest: None declared.

PO493-TUE

Impact of inherited risk factors of venous thromboembolism (VTE) on the associations between red cell distribution width and risk of VTE

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Background: Red cell distribution width (RDW), a measure of variability in size of circulating erythrocytes, is associated with future risk of venous thromboembolism (VTE) independent of traditional atherosclerotic risk factors. The underlying mechanism(s) remain unsettled. Shared genetic variants may be involved, but the joint effect of RDW and genetic thrombotic risk factors have not yet been studied.

Aims: To study whether the apparent association between RDW and VTE is inflicted by joint effect of genetic thrombotic risk factors and RDW in a case-cohort recruited from a general population.

Methods: Cases with a first ever VTE ($n = 639$) and an age-weighted subcohort ($n = 1730$) sampled from the Tromsø 4 (1994–95) study were included. DNA-samples obtained at baseline were genotyped for FV-Leiden (rs6025), F5 (rs4524), FGG (rs2066865), ABO blood type (rs8176719) and F11 (rs2036914 and rs2289252). Cox regression models with age as time-scale were used to calculate crude and multivariable adjusted hazard ratios (HRs) across categories of RDW and gene variants. The regional committee of research ethics approved the study and all subjects gave informed written consent.

Results: There were 639 incident VTEs during a median follow-up of 16 years. Subjects with RDW in the highest quartile had 26% higher risk of VTE than those in the lowest quartile (HR 1.26, 95% CI 1.00–1.57). The risk of VTE by RDW did not change after adjustment for the genetic variants. There was a moderately increased risk of VTE when the genetic variants F5 and non-O blood type were combined with high RDW. Compared to subjects with low RDW and no risk alleles, the HR of VTE by F5 increased from 2.03 (95% CI 0.95–4.33) for those with low RDW to 2.39 (95% CI 1.11–5.16) for those with high RDW.

Conclusion: Our findings suggest that the risk of VTE by high RDW is not caused by a joint effect of high RDW and genetic thrombotic risk factors.

Disclosure of Interest: None declared.

PO494-TUE

Impact of inherited risk factors of venous thrombosis on risk of stroke and VTE in atrial fibrillationRye-Holmboe I^{1,2}, Smith E^{3,4}, Hald E^{1,2}, Vik A^{1,2}, Brodin E^{1,2}, Brækkan S^{1,2}, Solomon T⁵, Wilsgaard T⁶, Rosendaal F⁷, Frazer K^{3,4} and Hansen JB^{1,2}

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Background: Atrial fibrillation (AF) is a well-known risk factor for stroke and has recently been identified as a risk factor for venous thromboembolism (VTE). Factor 5 is an essential co-factor in the coagulation cascade. Variants in the F5 gene (rs6025 [Factor V Leiden] and rs4524) are associated with an increased risk of VTE, and Factor V Leiden is associated with an increased risk of stroke. However, the impact on these genetics variants in the F5 gene on VTE risk in patients with AF has, to our knowledge, never been explored.

Aims: To investigate the joint effect of AF and F5 gene variants (rs6025 and rs4524) on VTE and stroke risks in a case-cohort recruited from a general population.

Methods: Cases with a first VTE ($n = 630$) and an age-weighted sub-cohort ($n = 1737$) sampled from the Tromsø 4 (1994–95) and 6 (2007–08) studies were included. Baseline DNA-samples were genotyped. Validated VTEs, stroke and AF were registered up to December 31st, 2010. Hazard ratios with 95% confidence intervals (CIs) for VTE and stroke were calculated across categories of AF and gene variants and compared to subjects without AF or the risk alleles. The study was approved by the regional committees for research ethics and all subjects gave their informed written consent.

Results: There were 224 subjects with AF and 252 with incident stroke during follow-up. The risk of incident VTE after a diagnosis of AF was increased by 2.3-fold (95% CI, 1.69–2.99). The risk was further increased in subjects carrying the rs6025 allele (HR 7.42; 95%CI, 3.67–15.00) and the rs4524 allele (HR 4.88; 95%CI, 2.95–8.06). Individuals with the presence of both variants had an even higher risk (HR 11.88; 95%CI, 5.37–26.30). We found an increased risk of AF-related stroke for subjects with rs6025 allele (HR 5.40; 95% CI, 1.72–17.00), but there was no such association with the rs4524.

Conclusion: Our findings imply that patients with atrial fibrillation carrying the F5 rs6025 and rs4524 variants are at higher risk of VTE.

Disclosure of Interest: None declared.

PO495-TUE

Impact of the fibrinogen gamma gene RS2066865 variant on the risk of venous thrombosis in cancer patientsDziewiecka O¹, Smith E^{2,3}, Brækkan S^{1,4}, Jensvoll H^{1,4}, Blix K^{1,4}, Solomon T⁵, Wilsgaard T⁶, Rosendaal F⁷, Frazer K^{2,3} and Hansen J-B^{1,4}

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Background: Venous thromboembolism (VTE) is a common complication in patients with malignancy. Family and twin studies show that up to two thirds of the VTE risk can be accounted for by genetic factors. However, since cancer patients are often excluded in genetic studies on VTE, limited information exists regarding the impact of inherited risk factors on VTE risk in these patients.

Aims: To study the prevalence of risk alleles at the known prothrombotic fibrinogen gamma gene (FGG) single nucleotide polymorphism (SNP) at rs2066865 and its associated VTE risk in cancer patients.

Methods: We conducted a case-control study in which the genotype of the FGG rs2066865 SNP was determined in 574 VTE cases and in 584 randomly selected control subjects matched for age and sex recruited from a general population (the Tromsø study). Logistic regression was used to calculate odds ratios (OR) for VTE. The study was approved by the regional committee for research ethics and all subjects provided informed written consent.

Results: The FGG rs2066865 risk alleles were detected in 520 individuals: 430 heterozygous and 90 homozygous among 1158 subjects. There were 322 patients with were diagnosed with cancer of which 200 had a VTE. Cancer was associated with a 2.0-fold (OR, 95% CI 1.6–2.7) higher VTE risk than cancer-free individuals. Logistic regression analyses with adjustment for age and sex showed that the risk of VTE was only marginally increased (OR 2.2, 95% CI 1.4–3.3) in cancer patients heterozygous for the FGG rs2066865 mutation. The risk was, however, considerably higher (OR 6.5, 95% CI 2.2–19.5) in homozygous individuals with cancer compared to cancer-free individuals without the mutation.

Conclusion: Risk alleles at the FGG rs2066865 SNP appear to further increase the risk of VTE in cancer patients, substantially so in homozygous individuals.

Disclosure of Interest: None declared.

PO496-TUE

Characteristics of hospital-associated thrombosis attributed to thromboprophylaxis failure

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Background: Hospital associated thrombosis (HAT) is a significant cause of patient morbidity and mortality. Thromboprophylaxis (TP) has been demonstrated in clinical trials to reduce venous thromboembolism (VTE) by two thirds in selected high risk patients. TP failure (HAT_{TPF}) is recognised but poorly understood.

Aims: To evaluate characteristics and risk factors for HAT_{TPF}.

Methods: Root cause analysis of HAT was established in April 2010. Characteristics of HAT_{TPF} were compared to events attributed to other causes over a 3 year period. HAT was considered to be HAT_{TPF} where patients with VTE risk factors and no bleeding risk factors were administered appropriate TP according to national guidance.

Results: of 476 fully investigated episodes, 205 (43.1%) were due to TPF. HAT_{TPF} manifested as PE in 46.1%. HAT_{TPF} followed medical, surgical and obstetric admission in 89 (43.6%), 107 (52.5%) and 8 (3.9%) respectively. Common risk factors were age ≥ 60 years (64.0%), reduced mobility for ≥ 3 days (57.8%), medical comorbidity (45.6%), surgery with significant reduction in mobility (24.0%), total anaesthetic and surgery time of > 90 min (22.5%) and dehydration (21.6%). HAT_{TPF} were more common post discharge compared to other HAT (45.2 vs. 31.3%, $P = 0.002$), with a median time to event from admission of 17 days (range 2 – 170). Mean number of risk factors was higher in HAT_{TPF} (3.1 vs. 2.8). Within surgical patients ($n = 245$), lower limb/pelvic surgery > 60 min (21.0 vs. 10.4%, $P = 0.023$), dehydration (13.3 vs. 5.2%, $P = 0.03$) and intra-abdominal inflammatory disease (11.2 vs. 3.6%, $P = 0.02$) were more prevalent in HAT_{TPF}. Within medical patients ($n = 216$), patients with HAT_{TPF} were significantly older (72.1 vs. 67.3 years, $P = 0.02$). Dehydration was more commonly associated with HAT_{TPF} (33.7% vs. 22.8%, $P = 0.08$), as was cardiorespiratory disease (30.0% vs. 19.0%, $P = 0.08$).

Conclusion: These findings suggest sub-groups of patients may benefit from intensified TP strategies and may inform risk stratification in future studies of extended TP in medical patients.

Disclosure of Interest: L. Roberts Grant/Research Support from: Covidien, Speaker Bureau of: Bayer PLC, Covidien, E. Gee: None declared, G. Mulla: None Declared, C. Doyle: None Declared, M. Ferreira: None declared, J. Patel Grant/Research Support from: Bayer PLC, R. Patel: None declared, R. Arya Grant/Research Support from: Bayer PLC & Covidien.

PO497-TUE

Anti-vimentin antibodies increase thrombus formation at venous shear rates *in vitro* and *in vivo*: implication for venous thromboembolism in autoimmune diseases

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Background: It is appreciated that patients with autoimmune diseases have an increased risk for a venous thromboembolism (VTE) event. Despite that autoantibodies against vimentin are well recognized, and that they may have the capacity to activate blood cells, their relevance to VTE remains unclear.

Aims: We investigated the effect of anti-vimentin antibodies (AVA) on thrombus formation *in vitro* at low shear rate or *in vivo* at venules.

Methods: We used AVA, and collagen fibrils. The *in vitro* assays to examine thrombus formation on a collagen surface were performed by perfusing whole blood at low shear rates (100–400 s⁻¹) using a micro-fluidic flow chamber. For *in vivo* thrombus formation, we have used a photochemical-injury model of thrombosis in the cremaster venules of wild-type mice and mice deficient in vimentin.

Results: The formation of microthrombi on collagen fibrils from human whole blood containing AVA was significantly ($> 25\%$) higher than blood mixed with isotype IgG. The occlusion time measured in venules of mice injected with AVA was significantly decreased (4.7 min, $n = 5$) as compared to animals treated with isotype IgG (7.3 min, $n = 5$) ($P = 0.02$). Interestingly, without AVA, vimentin-deficient mice had a decreased occlusion time (5.2 min, $n = 5$) as compared to wild type mice (8.9 min, $n = 5$) ($P = 0.02$). Moreover, thrombus formation on collagen fibrils was much higher (5 folds) when we perfused whole blood from vimentin deficient mice as compared to that of wild type mice at low shear rates.

Conclusion: AVA can induce thrombus formation at venous shear rates, implying a potential role in mediating VTE in patients with autoimmune diseases. Furthermore, the results from using vimentin deficient mice indicate that the lack of vimentin may increase the susceptibility of platelets for activation at low shear rates. We speculate that interaction of AVA with cell-surface vimentin impairs a biological role for vimentin in down regulating activation of platelets.

Disclosure of Interest: None declared.

PO498-TUE

Switching type of oral contraceptive and risk of venous thromboembolism: a case-crossover study

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Background: Oral contraceptives (OC) increase the risk of venous thromboembolism (VTE), where type of progestogen and dosage of estrogen are related to the size of the risk increase. For several reasons patients switch to a different type of OC. It is unknown if switching of OC type increases VTE risk.

Aims: To assess whether switching to a different OC increases VTE risk and to determine the effect of direction of change (i.e. to a type of pill with lower or higher VTE risk).

Methods: Case-crossover study. Premenopausal women with VTE aged < 50 years who used OC at time of VTE were selected from the MEGA-study. Detailed information on OC use was obtained by linkage to the Dutch Foundation for Pharmaceutical Statistics register. Conditional logistic regression models were used to estimate odds ratios (OR) with 95% CIs of switching of OC type in the 0–6 months period (risk period) before VTE onset compared with 3 different control periods (6–12 month, 12–18 months and 18–24 months prior to VTE onset).

Results: Prescriptions for 369 women with a first VTE were available. Of these, 118 patients used OC continuously in the preceding 24 months before VTE, of whom most ($> 89\%$) did not switch. Six patients switched in the risk period and 6 in control period 6–12 months, 4 in control period 12–18 months, and 5 in control period 18–24 months prior to VTE. The corresponding ORs were 1.0 (95% CI, 0.3–3.1), 1.5 (95% CI, 0.4–5.3) and 1.3 (95% CI, 0.3–6.0), respectively. Of the women who switched in the risk period, 5/6 (83%) switched to high risk OC vs. 5/15 (30%) in the control periods.

Conclusion: Switching type of OC does not appear to increase VTE risk substantially, although an effect of direction of change cannot be excluded. Larger studies are necessary to provide more conclusive evidence.

Disclosure of Interest: None declared.

PO499-TUE

'Forgotten no more' a case of a 54 year old female with Lemierre's syndrome

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Background: Lemierre's syndrome is a condition characterized by thrombophlebitis of the internal jugular vein and bacteremia caused by primarily anaerobic organisms, following a recent oropharyngeal infection. For reasons that are not clear, there has been an increase in the reporting of Lemierre's syndrome since the late 1990s

Aims: To be able to present a case report on a rare case of Lemierre's syndrome in the Philippine Heart Center

Methods: This is a case report done on a rare case of Lemierre's syndrome diagnosed at the Philippine Heart Center

Results: A 54 year old female who, initially presented with fever and associated neck pain, was later documented to have subperiosteal abscess and thrombosis of the right internal jugular vein, compatible with Lemierre's syndrome. Patient was treated with antimicrobial therapy and anticoagulation for a total of 6 weeks.

Conclusion: The typical clinical picture of Lemierre's syndrome is characteristic but many general practitioners are unaware of this condition and diagnosis is often delayed with potentially fatal complications. Successful management therefore rests on the awareness of the condition, a high index of suspicion, and a multidisciplinary team approach.

Disclosure of Interest: None declared.

PO500-TUE

Prevalence of risk factors predisposing to thrombosis in patients with central retinal vein occlusion

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Background: Retinal vascular occlusions are disorders that may lead to partial or complete blindness. Few and contrasting data are available on the prevalence of risk factors in patients with central retinal vein occlusion (CRVO).

Aims: The aim of our study was to establish the prevalence of major and potential inherited and acquired thrombophilic risk factors in 122 patients (65 females, 57 males; age: 57.2 years; range 23- 72) with CRVO. The control group consisted of 98 (50 females, 48 males; age: 56 years; range 19-73) healthy subjects, without any vascular, eye-related disease.

Methods: In all participants Leiden mutation (FV Leiden), hyperprothrombinemia (20210 G/A mutation), Leu 34 polymorphism of the factor (F) XIIIa-subunit, deficiency of protein C, S, antithrombin, hyperhomocysteinemia and antiphospholipid antibodies were assessed. Also intima-media thickness (IMT) in carotid arteries were measured using ultrasonography.

Results: In patients fasting homocysteine levels were significantly higher than in controls ($P < 0.05$). Among the remaining parameters tested, prevalence of FV Leiden, circulating antiphospholipid antibodies

and deficiency of protein C were significantly more common in CRVO patients ($P < 0.05$) compared with control group. There was no significant difference in the prevalence of deficiency of protein S, 20210 G/A mutation, FXIII Val34Leu polymorphism, antithrombin, and IMT between CRVO patients and controls. From classic risk factors - arterial hypertension, diabetes mellitus, hypercholesterolemia and cigarette smoking were significantly more frequent in patients with CRVO than among control subjects.

Conclusion: These data demonstrate a potential role of some classic and thrombophilic risk factors in the pathophysiology of CRVO. Measurement of these parameters may be useful in initiating appropriate management at the earliest.

Disclosure of Interest: None declared.

PO501-TUE

No differences in thrombin generation between patients with and without portal vein thrombosis in end-stage liver disease

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Background: Patients with End-Stage Liver disease (ESLD) have been considered to have a hypocoagulation tendency. During the last decade, hypercoagulation and thrombosis are being diagnosed more often in patients with ESLD due to the introduction of new tests such as rotational thromboelastometry (TEM).

Aims: Our aim was to investigate TEM changes in patients with ESLD and portal vein thrombosis (PVT).

Methods: 100 patients diagnosed with ESLD between January 2013-May 2014 were included in this study. PVT was diagnosed on abdominal contrast computer tomography. Patients were divided into two groups: patients with PVT (PVT group) and patients without PVT (non-PVT group). Demographic variables, ESLD severity scores (Model for ESLD – MELD and MELD-Sodium – MELD-Na), standard coagulation tests (prothrombin time – PT, international normalized ratio – INR, activated partial thromboplastin time – aPTT), serum fibrinogen (Clauss method) and platelet count were recorded. TEM (ExTEM, InTEM, ApTEM, FibTEM) were performed at the same time with standard coagulation tests. Both standard and derived coagulation parameters were noted.

Results: Fourteen patients (13.9%) were included in the PVT group and 87 patients in non-PVT group (86.1%). Age ($P = 0.520$), MELD ($P = 0.147$) and MELD-Na ($P = 0.336$) were similar between group. No differences in PT ($P = 0.983$), INR ($P = 0.254$), aPTT (0.403), Fibrinogen ($P = 0.634$) and platelet count ($P = 0.424$) were observed. Patients in PVT group had lower ExTEM maximum lysis ($P = 0.021$, 95% CI [-134.28, 11.9]) and increased ExTEM maximum clot firmness ($P = 0.018$, 95% CI [12.90, 131.90]). No statistical differences in thrombin generation, as shown by derived TEM parameters (potential index ($P = 0.128$), maximum velocity of clot formation ($P = 0.388$), time to maximum velocity of clot formation ($P = 0.957$), were observed.

Conclusion: Patients with PVT have decreased fibrinolysis and increased maximum clot firmness when compared with patients without PVT. No differences in thrombin generation were observed between the two groups.

Disclosure of Interest: None declared.

PO502-TUE

Endogenous sex hormone levels in men with unprovoked deep vein thrombosis

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Background: Venous thromboembolism (VTE) has a multifactorial etiology and the list of major risk factors include recent surgery or trauma, active cancer and prolonged immobilization for severe medical diseases. In women, the use of oral contraceptives and of hormone replacement therapies with estrogen and progesterone is associated with an increased risk of VTE. Conversely, the role of sex hormones as a risk factor for VTE in men is more controversial.

Aims: To assess the role of sex hormones in male patients with acute deep vein thrombosis (DVT).

Methods: Adult male patients with objectively diagnosed unprovoked DVT and sex matched control were prospectively included. Body mass index (BMI) was calculated in all the participants. Endogenous total testosterone (n.v. 2.8–11 ng mL⁻¹ for adult men) and estradiol (n.v. 11–44 pg mL⁻¹ for adult men) (converted from testosterone by an aromatase) were measured. Mean levels of endogenous sex hormones in case and controls were compared. Furthermore the risk of DVT for each tertile of testosterone levels was evaluated calculating the odds ratios (OR) with the corresponding 95% confidence interval (CI) using the lowest tertile as reference.

Results: Sixty-three DVT patients and 63 controls were included. Mean serum testosterone and estradiol levels were similar (3.9 vs. 4.0 ng mL⁻¹ and 28.6 vs. 30.2 pg mL⁻¹ respectively; *p* NS). The analysis that considered different tertiles of testosterone levels also failed to identify any significant association with the risk of DVT (OR 1.65, 95% CI 0.70, 3.87 and 1.06, 95% CI 0.43, 2.60 respectively for the second and third tertile compared to the lowest tertile).

Conclusion: In our study, sex hormones levels were similar in male patients with an acute episode of unprovoked DVT in comparison to sex and age matched controls. Thus, testosterone and estradiol should not be routinely tested in patients with unprovoked events.

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PO503-TUE

A-fibrinogen THR312ALA polymorphism is an independent genetic risk factor for venous thromboembolism in the population of north-western Russia

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Background: The role of genetic mechanisms in most cases of venous thromboembolism (VT) is still unclear. Fibrinogen Aα (FI-A) Thr312Ala polymorphism has been shown to influence factor XIII-dependent cross-linking and fibrin clot structure. Moreover, in some population groups it was associated with increased risk of VT.

Aims: To evaluate the role of FI-A Thr312Ala polymorphism as a risk factor for VT in the population of North-Western Russia.

Methods: Retrospective study involved 300 patients with VT (147 men and 153 women, mean age 42.3 ± 12.6 years) and 243 sex- and age-matched healthy controls (HC). All individuals originated from the North-Western region of Russia and gave informed consent for participation in the study. FI-A Thr312Ala polymorphism was discriminated by PCR-RFLP technique. The differences in genotype distributions between groups were estimated by Fisher's exact test.

Results: Homozygosity for the FI-A Ala312 variant was more frequently seen in patients compared to HC group (13.7% vs. 4.9%; OR=3.0; 95% CI: 1.6–5.9; *P* = 0.0007). This genetic factor was highly prevalent in both men (12.9%) and women (14.4%) with VT. In the group of 232 patients having neither FV Leiden nor FII G20210A mutation, the FI-A 312 Ala/Ala genotype was present in 28 (12.1%) cases (OR=2.6; 95% CI: 1.3–5.3; *P* = 0.008, compared to HC). Finally, homozygosity for the FI-A Ala312 variant occurred more frequently in the group of 200 patients with early-onset disease (at age 45 or less) than in those who suffered from first VT episode after 45 years old (16.0% vs. 9.0%, respectively; OR=1.9; 95% CI: 0.9–4.2; *P* = 0.11). When compared to HC group, the prevalence of the FI-A 312 Ala/Ala genotype has been proved to be a significant risk factor for VT development in young patients originated from the North-Western region of Russia.

Conclusion: We suggest that FI-A Thr312Ala polymorphism is an independent genetic risk factor for VT in the population of North-Western Russia.

Disclosure of Interest: None declared.

PO504-TUE

Venous thromboembolism (VTE) in hospitalised non-surgical cancer patients and its burden on healthcare in an Asian tertiary hospital

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Background: Despite rising trends of VTE in Asian hospitals, information on cancer patients with VTE here is scarce.

Aims: To determine the prevalence of VTE in hospitalised medical patients with cancer, identify the risks of VTE and assess its impact on healthcare burden.

Methods: The Singapore General Hospital discharge database from 2004 to 2011 was reviewed to identify medical patients with cancer, deep vein thrombosis (DVT), pulmonary embolism (PE) and other comorbid conditions using the Australian Modification (ICD-9-AM) codes in International Statistical Classification of Diseases and Related Health Problems. The cancer types, impact of VTE on the mortality, readmission rates and length of hospitalisation were analysed. Logistic regression analyses and multivariate regression odds ratio (OR) analyses were used to adjust for age, gender, ethnicity, and other co-morbidities.

Results: One thousand and three hundred and two (8.7%) of 14,969 non-surgical cancer patients identified had VTE. Gastrointestinal, haematological, ovarian and lung malignancies were most at risk of developing VTE (OR > 2). Metastatic disease significantly increased the rates of VTE (10.8% vs. 6.5%) for both DVT (7.7% vs. 5.0%) and PE (4.1% vs. 1.9%). Metastasis was a significant and independent predictor of VTE (OR 2.2, 95% CI 2.0–2.5) by logistic regression analyses. The mortality rates for patients with no cancer nor VTE, VTE only (VTE), cancer only (Ca) and both cancer and VTE (Ca/VTE) were 1.7%, 7.2%, 8.7%, and 18.5% (*P* < 0.001) respectively. Multivariate analysis indicated that the OR for Ca/VTE, Ca and VTE were 18.4,

8.4 and 3.2 respectively. 30-day unscheduled readmission OR were increased with Ca/VTE (4.2), Ca (3.4) and VTE (1.9). Hospital length of stay incidence-rate ratio (IRR) were increased with Ca/VTE (4.9), Ca (2.4) and VTE (4.5).

Conclusion: Cancer increases the risk of VTE which significantly impacted on healthcare burden with increased mortality, length of hospital stay and unscheduled readmission rates. Further studies on VTE prophylaxis should be considered.

Disclosure of Interest: None declared.

PO505-TUE

A novel microfluidic device development for venous thrombus understanding

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Background: Pulmonary thromboembolism sometimes causes sudden death. Prevention is hard for lacking mechanism understanding. There are much fewer previous venous thrombus studies than arterial one.

Aims: Our aim is to extract physical elements of randomness and reproducibility of venous thrombus formation as convective reaction-diffusion system.

Methods: To meet our aim, we made a microfluidic device of silicone rubber and a cover glass to visualize and quantify the thrombus formation with spatial, time and fluid field information. The inside was fully siliconized and partly coated with collagen and temperature was controlled. Fluorescently-stained blood was flowed with venous shear rate by a pressure control pumping device. We imaged the thrombus formation by high-speed camera.

Results:

- 1 The length of collagen-coated part correlated with size of thrombus.
- 2 Temperature significantly influenced thrombus formation.
- 3 Temperature influenced the plasma viscosity much more than hematocrit.
- 4 There were some reproducible patterns in the motion and the positional relation of fibrin, platelets, hemocytes and other elements.
- 5 Small fluorescent particles could be observed in fluid field.

Conclusion: Basically our element technologies of microfluidics can be useful to quantify the thrombus formation with spatial, time and fluid field information. It will be helpful for understanding the mechanism of venous thromboembolism as convective reaction-diffusion system. Fluid field will be analyzed in particle image velocimetry by our imaging technology. To bring more physiological conditions, we have some improvement plans such as lining the inside with endothelial cells, designing another fluidic device of round-shaped cross section, and so on. Above technologies should be useful to develop a mathematical model for calculating venous thrombogenesis in future. We made a novel microfluidic device to visualize and quantify the venous thrombus formation. It will be helpful for understanding the mechanism of venous thromboembolism.

Disclosure of Interest: None declared.

PO506-TUE

Anticoagulants for prevention of thrombotic complications in patients on parenteral nutrition: a systematic review

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Background: Patients on parenteral nutrition (PN) for intestinal failure require central venous access, which is associated with the risk of catheter-related thrombosis (CRT) and pulmonary embolism (PE). There is poor consensus on CRT thromboprophylaxis, whilst comorbidities often represent a contraindication for anticoagulants.

Aims: The aim of this review is to systematically evaluate the effect of anticoagulation for CRT prevention in patients receiving PN.

Methods: We performed a systematic literature search. Intervention and observational studies regarding either adult or pediatric patients on PN receiving anticoagulant regimens were included. Primary outcomes were: the rates of objectively confirmed CRT, PE, and major bleeding events. Secondary outcomes were: prevalence, pharmacokinetics, and cost-effectiveness of anticoagulant agents, and rate of heparin-induced thrombocytopenia.

Results: We identified 1125 studies, of which 22 were included (7 intervention studies). None of the intervention studies showed an effect of prophylactic unfractionated heparin for primary CRT prevention, while observational studies showed heterogeneous results. No studies involved patients with acute CRT. Data regarding the rates of other complications were scarce. The prevalence of anticoagulant administration varied, ranging from 22 to 54% in studies published during the past decade.

Conclusion: The administration of anticoagulants for CRT prevention in patients on PN is not supported by evidence. Although anticoagulation is often prescribed, crucial gaps of knowledge regard efficacy and safety of anticoagulants in this specific population, as well as their pharmacokinetics and quality of treatment.

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PO507-TUE

Long-term parenteral nutrition-associated thromboembolic and hemorrhagic complications in 236 single-center outpatients

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Background: Prevalence of home parenteral nutrition (PN) in Europe is 1–13/million inhabitants. Although patients on PN require central venous access and are often anticoagulated, little information is available regarding the rates of catheter-related thrombosis (CRT), pulmonary embolism (PE), major bleeding, heparin-induced thrombocytopenia (HIT), and vena cava syndrome (VCS).

Aims: The main objective of this preliminary analysis is to describe the rates of thrombotic/bleeding complications in patients on home PN.

Methods: We performed a longitudinal retrospective study by chart review of consecutive patients on home long-term PN followed at our tertiary academic institution (1994–2014). We analyzed the absolute risk of first and recurrent thromboembolic and bleeding events. All events were independently adjudicated. Data are presented as PN-time risk (n of patients with events/total N , 95% confidence interval, 95 CI) and incidence rate (number of events/patient-year, 95 CI).

Results: Two-hundred-thirty-six patients were included (male sex 40%, short bowel syndrome 58.1%, cancer 27.5%, prior thrombosis 39.8%) and followed for a total of 690 patient-years (median 17 months, interquartile range: 8–43). Systemic anticoagulation was prescribed to 146 (62%) patients at PN onset, and to 177 (75%) patients during PN (445 patient-years). Fifty symptomatic objectively-diagnosed CRT events occurred in 36 patients (20.3%, 95 CI 15.6–25.8; 7.2/100 patient-years, 95 CI 5.5–9.6), while 18 patients had PE (7.6%, 95 CI 4.7–11.6; 2.6/100 patient-years, 95 CI 1.6–4.0). Thirty-two patients had at least one major bleeding (13.6%, 95 CI 9.6–18.4; 5.8/patient-years; 95 CI 4.2–7.8). Medium-high probability HIT occurred in one patient (0.6/100-heparin treatments, 95 CI 0.03–2.9). VCS was diagnosed in 7 patients (3.0%, 95 CI 1.3–5.8).

Conclusion: Thrombotic and bleeding events were frequently complicating PN administration in our cohort of patients: complications rates occurred more frequently than in other non-PN populations described in literature.

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PO508-TUE

Role of body composition measurements in predicting cancer associated thrombosis

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Background: Body composition measurements have been associated with increased risk of thrombosis. Venous thromboembolism (VTE) is one of the complications of cancer and significantly effects morbidity and mortality.

Aims: To evaluate body composition measurements including height, body surface area (BSA), sarcopenia, body mass index (BMI) and lean body mass (LBM) on the risk of cancer associated VTE.

Methods: From a prospectively maintained database of pre-chemotherapy patients with cancer, we retrospectively reviewed 185 individuals with abdominal CT available within 3 mo of recruitment. Cancer specific variables as well as basic demographic data were available. VTE events had been independently adjudicated. Body surface was derived using the Mosteller equation. LBM was derived: (L3 muscle area in cm² – 3.2459)/3.0583. Height was categorized at the gender specific 3 quartile by gender. Sarcopenia was defined from an L3 level muscle area divided by height, using validated cutoff (53.4 male; 38.5 female); the rest of the variables were categorized by quartile.

Results: There were no missing data on the selected 185 patients. The patients were 60 yo (median), 70% were female, 35% had metastatic

disease at baseline, 34% had gynecological malignancies, 17% breast, 14% pancreatic cancer. The median follow up was 11.9 mo. The majority were intermediate Khorana score (63%), 17% died and 14% developed a VTE. Taller patients had a higher likelihood of VTE [Hazard ratio (HR): 2.8 95%Confidence Interval (95 CI) 1.3 - 6.2]. The association remained significant after controlling for Khorana score [HR 2.9; 95 CI 1.3 - 6.4], metastatic disease [HR 2.9; 95 CI 1.3 - 6.3] and ECOG status [HR 2.7; 95 CI 1.2 - 6.1]. BMI, BSA, sarcopenia, LBM were not significantly associated with VTE.

Conclusion: As reported in non-cancer population, height appears to have a predictive value in the occurrence of cancer associated VTE.

Disclosure of Interest: None declared.

PO508.1-TUE

A novel RS2232710 single nucleotide variant in the protein z-dependent protease inhibitor (ZPI, SERPINA10) gene and a risk of deep vein thrombosis

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Background: Rare mutations in PROC, PROS1 or SERPINC1 as well as common polymorphisms in F5, F2, CYP4V2, SERPINC1, GP6 or HIVEP1 have been recently identified as risk factors for deep vein thrombosis (DVT). To identify novel genetic risk factors for DVT, we have developed and successfully applied the next-generation DNA sequencing (NGS) and the data analysis pipeline of the coding area of 186 hemostatic/proinflammatory genes in Italian idiopathic DVT cases and controls. Using this strategy, we identified a single nucleotide variant (SNV) rs6050 in FGA and an excess of rare SNVs in ADAMTS13 associated with DVT. Our NGS-based data analysis revealed additional 18 novel variants with putative role in DVT.

Aims: To replicate the 18 novel variants identified in our NGS study.

Methods: All variants were genotyped by Sanger sequencing in a cohort of up to 719 Italian idiopathic DVT patients and 719 healthy controls. We identified rs2232710 SNV in the protein Z-dependent protease inhibitor (ZPI/SERPINA10) gene as a risk factor for DVT in the Italian population. To confirm this association, we performed TaqMan SNP genotyping replication in two independent Dutch cohorts: the LETS study (454 idiopathic DVT cases and 451 controls) and the MEGA study (3799 idiopathic DVT cases and 4399 controls).

Results: We identified a novel SNV rs2232710 in the ZPI gene to be associated with an increased risk of DVT with odds ratio of 2.74 (95% CI 1.33–5.65; P value = 0.0045). The rs2232710 is a low frequency variant in the Italian replication cohort and results in missense change of Gln384Arg that has been predicted by SIFT and Mutation Taster databases as potentially damaging. However, the rs2232710 SNV showed no association with DVT in the two independent Dutch replication cohorts.

Conclusion: We found a novel, non-synonymous rs2232710 variant in the ZPI gene associated with DVT in the Italian population. However, replication in two independent Dutch cohorts did not confirm this association.

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Signal Transduction

PO509-TUE

Urokinase plasminogen activator protects cardiac myocytes from oxidative damage and apoptosis via OGG1

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Background: The role of uPA in tissue remodeling and cell migration is already well established. In addition, uPA was reported to stabilize p53, a key cell cycle control, DNA repair and apoptosis initiation protein.

Aims: To investigate the role of uPA-uPAR signaling towards cell survival or apoptosis in human adult cardiac myocytes (HACM).

Methods: HACM were isolated from failing human hearts. DNA damage was inflicted by incubating cells for 2 h with 200 µM H₂O₂. To analyze for apoptotic cells we applied TUNEL (TdT-mediated dUTP-biotin nick end labeling) staining. Oxidative damage foci were analyzed by staining for 8-oxoguanine base pairs using a monoclonal antibody.

Results: We did not observe an effect of uPA on cell survival in baseline conditions. However, when HACM were treated with H₂O₂ after 24 h of preincubation with uPA we found a reduction of apoptotic cells (57% apoptotic cells in control conditions vs. 42% apoptotic cells in uPA pretreated conditions, $P = 0.003$). In addition, oxidative DNA damage was reduced significantly by 59% ($P = 0.05$) as indicated by a reduction of 8-oxoguanine foci in HACM nuclei. Protection from apoptosis was independent from uPA catalytic activity, as the amino-terminal fragment of uPA (ATF) displayed a similar anti-apoptotic effect (70% apoptotic cells in control conditions vs. 40% apoptotic cells in ATF pretreated conditions, $P < 0.05$). One of the main proteins to repair oxidative DNA damage is the p53 induced protein OGG1. We found a significant increase of OGG1 in HACM pretreated with uPA (1.4 fold induction, $P = 0.02$). Knockdown of OGG1 resulted in a complete abrogation of the protective effect of uPA (12% under control conditions vs. 62% apoptotic cells under knockout conditions, $P = 0.01$).

Conclusion: We conclude that uPA might have a tissue protective role in human hearts besides its role in tissue remodeling. This tissue protection is mediated by the DNA repair protein OGG1.

Disclosure of Interest: None declared.

PO510-TUE

Factor VII activating protease (FSAP) expression is negatively regulated by transforming growth factor-beta (TGF-β) in the liver

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Background: Human population genetic studies as well as experiments with FSAP-deficient mice show that FSAP is a regulator of pathways leading to thrombosis and stroke. FSAP is a circulating serine protease, mainly expressed and secreted from the liver. Plasma FSAP antigen levels have been shown to be increased in diverse conditions such as pregnancy and stroke. However, mechanisms regulating the concentration of FSAP in the blood remain unknown.

Aims: The aim of our study is to investigate the regulation of FSAP expression in primary mouse hepatocytes and a mouse hepatocytic cell line (AMI-12) by identifying the signaling pathway involved.

Methods: Expression at the mRNA level was studied by qPCR and proteins by Western Blotting. Promoter reporter studies and chromatin immuno-precipitation assays (CHIP) were performed to identify relevant transcription factors.

Results: Of all stimuli that were tested, including cytokines, growth factors and hormones, TGF-β showed the most dramatic effect. Treatment with TGF-β caused a 50% reduction of FSAP mRNA and protein levels of secreted FSAP after 24 h. This effect was blocked by the TGF-β1-receptor (Alk-5) antagonist SB431542 but not by SIS3 which inhibits Smad3. Luciferase promoter assays identified a TGF-β responsive region proximate to the FSAP gene at position -80 to -500 relative to the transcription start site of the FSAP gene. Subsequent *In-silico* analysis of this region identified the activator protein 1 complex (AP-1) as the most likely candidate transcription factor involved in this regulation. Chromatin immuno-precipitation assays confirmed reduced AP-1 binding upon TGF-β treatment.

Conclusion: Thus, TGF-β, a key factor regulating liver fibrosis and the development of hepatocellular carcinoma, is a strong down regulator of FSAP expression. Liver dysfunction is often related to abnormal production of hemostasis factors and we suggest that FSAP expression will be altered in such states contributing to altered hemostasis and thrombosis.

Disclosure of Interest: None declared.

PO511-TUE

Phosphatidylinositol-3,4,5-trisphosphate stimulates Ca²⁺ elevation and AKT phosphorylation to constitute a major mechanism of thromboxane A2 formation in human platelets

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Background: Phosphatidylinositol(3,4,5)trisphosphate (PIP₃) is formed from phosphatidylinositol(4,5)bisphosphate (PIP₂) by PI-3kinase. It has been implicated in many platelet functions however many of the mechanisms need clarification

Aims: We have used cell permeable analogues of PIP₃, 1-O-(1,2-di-palmitoyl-sn-glycero-3-O-phosphoryl)-D-myo-inositol-3,4,5-trisphosphate (DiC16-PIP₃) or 1-O-(1,2-di-octanoyl-sn-glycero-3-O-phosphoryl)-D-myo-inositol-3,4,5-trisphosphate (DiC8-PIP₃) to study their effects on activation of freshly prepared washed human platelets.

Methods: Washed human platelets were isolated from freshly donated blood for aggregation studies, labeled with Fura2 for Ca^{2+} studies, or prepared for western blotting for detection of Akt phosphorylation, PLC γ 2 phosphorylation, syk phosphorylation or detection of thromboxane B₂ formation.

Results: Addition of either DiC8- or DiC16-PIP₃ to human platelets induced aggregation in the presence of extracellular Ca^{2+} . This was reduced by the presence of indomethacin, the phospholipase C inhibitor U73122 and 2-aminoethyl diphenylborinate (2APB). DiC8-PIP₃ induced the phosphorylation of Akt which was reduced by the Akt inhibitor IV and EGTA suggesting a dependence on Ca^{2+} entry. In Fura2 loaded platelets DiC8-PIP₃ was effective at increasing intracellular Ca^{2+} in a distinct and transient manner that was reduced in the presence of indomethacin, U73122 and 2APB. Ca^{2+} elevation was reduced by LOE908 and by BTP2. DiC8-PIP₃ induced the release of Ca^{2+} from stores which was not affected by bafilomycin A1 and was more potent than the two pore channel agonist DiC8-PI(3,5)P₂ suggesting release from an endoplasmic reticulum type store. Finally like thrombin DiC8-PIP₃ induced the formation of thromboxane B₂ that was inhibited by the Akt inhibitor IV.

Conclusion: These studies suggest that PI(3,4,5)P₃ via Ca^{2+} elevation and Akt phosphorylation forms a central role in thromboxane A₂ formation and the amplification platelet activation.

Disclosure of Interest: None declared.

PO512-TUE

A systems biology model for the coagulation network describes biomarker changes observed in a clinical study with FVIIA variant

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Background: PF-05280602 (PF-602) is a variant of activated recombinant human factor VII (FVIIa), currently in development for treatment and prevention of bleeding in hemophilia A or B patients with inhibitors. Preliminary pharmacokinetic (PK) and pharmacodynamic (PD) data for the phase 1 study of single dose escalation of PF-602 in hemophilia patients are available. To enable integrated understanding of all the data and underlying pharmacology, our group has implemented a quantitative systems pharmacology (QSP) model for the coagulation pathway. The model was optimized to describe *in vitro* data of thrombin generation assay (TGA), aPTT and PT as well as *in vivo* prothrombin fragment 1 + 2 (PF1 + 2) changes from clinical studies with Eptacog Alfa.

Aims: To use the QSP model to test whether increased catalytic reaction rates for PF-602 compared with Eptacog Alfa translate to changes in the observed biomarker responses.

Methods: Single IV infusion of PF-602 and Eptacog Alfa at multiple dose levels were simulated in the QSP model. In the model, Eptacog Alfa was assumed to have the same kinetics as endogenous FVIIa and PF-602 has increased reaction rates for both tissue factor-dependent and -independent activation of FX into FXa. The PK property observed clinically for each molecule was used in the model. The biomarkers that the model simulated include TGA, aPTT, PT, PF1 + 2 and thrombin-anti-thrombin III complex.

Results: The model was able to describe dose-dependent clinical biomarker data with PF-602 well. Since no biomarker data for Eptacog Alfa were available from the same study, we used the model simulation to demonstrate a left-shift of the biomarker dose-response curve for PF-602 compared with Eptacog Alfa.

Conclusion: The model simulation results suggest that our understanding of the increased kinetic rates for PF-602, taken together with the PK profile, is sufficient to explain observed biomarker changes.

Disclosure of Interest: D. Lee Employee of: Pfizer Inc., S. Nayak Employee of: Pfizer Inc., S. Arkin Employee of: Pfizer Inc., S. Martin Employee of: Pfizer Inc., A. Heatherington Employee of: Pfizer Inc., P. Vicini Employee of: Pfizer Inc., F. Hua Employee of: Pfizer Inc.

PO513-TUE

Importance of transforming growth factor β -induced signaling for chronic postthrombotic vascular remodeling processes

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Background: Chronic Thromboembolic Pulmonary Hypertension (CTEPH) represents a misguided chronic fibrotic response to unresolved pulmonary thrombi. However, the pathomechanisms underlying thrombofibrotic pulmonary artery stenosis are largely unknown.

Aims: To investigate the role of TGF β , released from activated platelets, during CTEPH and to determine whether it promotes pulmonary fibrosis via endothelial-to-mesenchymal transition (EndMT).

Methods: Human endarterectomy specimens are processed for histological analysis (including Tissue MicroArrays) and quantitative *real time* PCR (qPCR). Cells from CTEPH tissue are *ex vivo* expanded. Findings in humans are compared to murine venous thrombi at different time points after *Vena cava inferior* (VCI) ligation.

Results: To confirm the presence of EndMT, immunofluorescence staining was performed on cryo-preserved CTEPH tissue and out-grown cells demonstrating cells simultaneously expressing endothelial (VE-cadherin, CD31) and mesenchymal (SMA, FSP1) markers. Also, the EndMT transcription regulators Snail and Twist were highly expressed on the mRNA level. Immunohistochemistry and qPCR confirmed the expression of TGF β 1 and several known TGF β receptors (i.e. TGFBR1, endoglin, BMPRII, ALK1 and ALK5). Areas rich in endothelial cells and/or myofibroblasts were strongly positive for phospho-SMAD2 and phospho-SMAD5, confirming active TGF β signaling, and similar findings were obtained in murine venous thrombi. To determine the causal role of TGF β during chronic post-thrombotic remodeling processes, mice with platelet-specific TGF β deletion (P4.Cre x TGF β 1^{flox/flox}) and their littermate controls are subjected to VCI ligation followed by ultrasound and histological examination of venous thrombi over 3 weeks.

Conclusion: Our analysis in CTEPH patient material and murine venous thrombi suggest that TGF β -induced signaling contributes to post-thrombotic vascular remodeling processes and fibrosis, possibly by promoting endothelial phenotype conversion.

Disclosure of Interest: None declared.

PO514-TUE

Role of P110alpha isoform of class I PI3-kinases in platelet activation and functions

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Background: Class I phosphoinositide 3-kinases (PI3Ks) are important signaling enzymes in the process of blood platelet activation. Class IA

PI3K beta has been proposed as a potential drug target to treat arterial thrombosis, a major cause of death worldwide. We recently showed that platelet class I PI3K beta is not essential for thrombus growth and stability at normal arterial shear but has a specific and critical role in maintaining the integrity of the formed thrombus upon elevation of shear rate. Our data show that absence of class I PI3K beta cannot be compensated by class I PI3K alpha. Although the role of PI3K alpha is still poorly characterized in platelets, studies using pharmacological inhibitors suggest that both PI3K alpha and beta are required, in a non redundant way, for full platelet activation by collagen. PI3K alpha has also been involved in IGF-1-mediated potentiation of platelet function.

Aims: To precisely characterize the role of PI3K alpha in platelet activation *in vitro*, *ex vivo* and *in vivo*, we generated a mice line expressing an inactive form of p110 alpha selectively in the megakaryocyte lineage.

Methods: A series of specific tests including measurements of PtdIns (3,4,5)P₃ production, light transmission platelet aggregometry, thrombus formation *ex-vivo* under flow conditions and *in vivo* arterial thrombosis models were performed.

Results: The data indicated a precise and selective contribution of PI3K alpha in platelet activation and thrombus formation.

Conclusion: Since class I PI3K alpha is frequently mutated in cancer and selective inhibitors are under development as antitumor drug, these results may help to anticipate the potential side effects of such treatment on haemostasis.

Disclosure of Interest: None declared.

Stroke

PO515-TUE

Evaluation of the therapeutic effect of novel stroke drug SMTP-7 on embolic stroke in monkeys

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Background: Tissue plasminogen activator (t-PA)-based thrombolytic therapy is a powerful means for the treatment of acute ischemic stroke, whereas it causes ischemia/reperfusion injury, occasionally leading to cerebral inflammation and hemorrhagic transformation, which result in limited therapeutic time window (TTW). Therefore, an alternative drug that overcomes these drawbacks is urgently needed. SMTP congeners are a promising stroke drug candidate, one of which is under clinical development. The excellent activity shown *in vivo* is attributable to a thrombolytic activity in conjunction with an anti-inflammatory activity via inhibition of soluble epoxide hydrolase (sEH).

Aims: Our study aims at 1) evaluating efficacy of SMTP-7 in an embolic stroke model in monkeys, and 2) identification of pharmacological rationale.

Methods: In an embolic stroke model induced by injection of blood clot, either saline, SMTP-7 (10 mg kg⁻¹, i.v.) or t-PA (3 mg kg⁻¹, i.v.) was injected 3 h after the embolization, and plasma and brain tissue slice were collected after additional 21 h. Infarction was evaluated by TTC- and HE-stained brain sections. Changes in pro-inflammatory cytokines, chemokines, enzymes involved in tissue proteolysis and fibrinolytic parameters, as well as levels of eicosanoids in plasma were measured.

Results: SMTP-7 reduced infarct volume compared to saline-treated group (by 65%, $P = 0.028$). An increase in plasmin- α_2 -antiplasmin level (6.1-fold, $P = 0.049$) and reduction in MMP-9 ($P = 0.035$) were also observed in the SMTP-7-treated animals. Eicosanoid analysis revealed that plasma dihydroxyeicosatrienoic acids, a fatty acid dioxides metabolized by sEH, was significantly reduced by the SMTP-7 treatment (by 40.3%, $P = 0.043$), suggesting intrinsic inhibition of sEH.

Conclusion: SMTP-7 effectively treats serious embolic stroke in monkeys. This is attributable, at least in part, to its unique profibrinolytic property along with anti-inflammatory/tissue-protective effects mediated by sEH inhibition.

Disclosure of Interest: None declared.

PO516-TUE

The association of factor VIII and von Willebrand factor levels and the outcome of thrombolysis in ischemic stroke patients

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Background: Thrombolysis by recombinant tissue plasminogen activator (rtPA) is an effective therapy in acute ischemic stroke (IS).

Aims: To investigate whether levels of factor VIII (FVIII) and von Willebrand factor (VWF) affect the outcome of thrombolysis.

Methods: Study population included 132 consecutive IS patients, who underwent thrombolytic therapy within 4.5 h after the onset of IS symptoms. Blood samples were taken on admission, 1 h and 24 h after the administration of rtPA. FVIII activity using chromogenic assay and VWF antigen levels were measured from all blood samples. The National Institutes of Health Stroke Scale (NIHSS) score and results of imaging tests were registered on admission, day 1 and 7 after therapy. Long term outcome was assessed at 3 months after the event by the modified Rankin scale. The study was approved by the Regional Ethics Committee and informed consent was obtained.

Results: FVIII and VWF levels on admission were significantly elevated in case of severe IS (NIHSS > 15) as compared to less severe IS (NIHSS < 5). This association remained significant after adjustment to CRP. FVIII levels dropped significantly 1 h after the initiation of rtPA infusion and showed an increase 24 h after thrombolysis, while VWF levels remained elevated during the course of thrombolytic therapy. The two parameters showed good correlation on admission but no significant correlation was found 1 h after thrombolysis, most probably due to plasmin-mediated FVIII degradation. No association was found between FVIII/VWF levels and therapy-associated hemorrhagic complications. Elevated FVIII/VWF levels 24 h after thrombolysis were associated with poor short-term and long-term functional outcomes and death.

Conclusion: Elevated FVIII/VWF levels on admission are associated with more severe stroke. Elevated FVIII/VWF levels 24 h after thrombolysis predict poor functional outcomes and are associated with mortality.

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Disclosure of Interest: None declared.

PO517-TUE

ADAMTS13 destabilizes thrombi in a mouse model of thrombotic focal cerebral ischemia

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Background: Currently, thrombolysis using tissue-plasminogen activator is the only treatment option in acute ischemic stroke, but it has

serious limitations. By cleaving von Willebrand factor, the metalloprotease ADAMTS13 has both antithrombotic and anti-inflammatory properties.

Aims: In this study, we analyzed whether ADAMTS13 can promote thrombus destabilization in the acute setting of ischemic stroke.

Methods: We used a murine stroke model in which an occluding platelet rich thrombus is generated in the right middle cerebral artery (MCA) by topical FeCl₃ application. Two types of injury were used: a threshold injury after which the endogenous thrombolytic system is able to induce spontaneous recanalization and a severe injury, which results in permanent MCA occlusion.

Results: After threshold MCA injury, spontaneous recanalization was observed within the first hour after occlusion in WT animals. In contrast, spontaneous recanalization was significantly impaired in ADAMTS13 KO mice ($P < 0.01$). As a result, cerebral infarct sizes 24 h post occlusion were significantly larger in ADAMTS13 KO mice when compared to WT mice ($11.9 \pm 1.9 \text{ mm}^3$ and $4.1 \pm 1.6 \text{ mm}^3$, respectively; $P < 0.01$). Interestingly, administration of recombinant ADAMTS13 (rADAMTS13) to ADAMTS13 KO mice 5 min after occlusion restored MCA blood flow. This also led to reduced cerebral infarctions, similar to WT mice. Severe MCA injury led to stable thrombus formation in all WT mice without spontaneous recanalization. Interestingly, also in this model, administration of rADAMTS13, 5 min after vessel occlusion, restored MCA blood flow and significantly reduced infarct size in a dose dependent way ($P < 0.01$). Even when rADAMTS13 was administered 60 min after occlusion, MCA blood flow was still partly restored, significantly reducing cerebral infarctions ($11.3 \pm 1.5 \text{ mm}^3$ compared with $18.8 \pm 2.9 \text{ mm}^3$ for vehicle treatment, $P < 0.05$).

Conclusion: ADAMTS13 destabilizes occluding intracranial thrombi and may have prothrombotic potential for the treatment of acute ischemic stroke.

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PO518-TUE

Remote platelet function testing using measurements of P-selectin in patients with acute stroke or TIA already receiving treatment with antiplatelet agents

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Background: Patients with acute stroke or TIA are prescribed antiplatelet agents. TARDIS is an international multicentre trial of patients with acute stroke or TIA, and is testing the safety and efficacy of intensive antiplatelet agents (combined aspirin, dipyridamole and clopidogrel) vs. guideline (aspirin and dipyridamole, or clopidogrel alone) given for 1 month. In a substudy, platelet function is measured at baseline and after 7 days.

Aims: To assess the effectiveness of pre-existing antiplatelet treatment in patients with acute stroke or TIA at baseline prior to randomisation.

Methods: Platelet function testing was via measurement of P-selectin on platelets using the Aspirin and P2Y₁₂ Test Kits from Platelet Solutions Ltd. Blood samples were stimulated with arachidonic acid or adenosine diphosphate immediately after collection, stabilised with PAMFix (from PSL) and sent by post to Nottingham for flow cytometric analysis.

Results:

	A	AD	C	2p A+AD v C
Aspirin Test (n)	125	14	16	
% P-selectin +ve, mean (SD)	27 (16)	25 (16)	47 (28)	0.003
MF, median [IQR]	166 [131, 223]	159 [137, 272]	257 [157, 775]	0.012
P2Y ₁₂ Test (n)	134	20	16	
% P-selectin +ve, mean (SD)	81 (13)	73 (23)	63 (21)	< 0.001
MF, median [IQR]	1022 [772, 1233]	1008 [570, 1294]	585 [352, 906]	< 0.001

The data for the Aspirin Test shows that the group taking aspirin (A) alone or in combination with dipyridamole (AD) had low platelet function; however 5% of patients had a level that remained high (median fluorescence, MF > 500). The P2Y₁₂ Test shows that those taking clopidogrel (C) had low platelet function; however 44% of these had a level that remained high (MF > 500).

Conclusion: Patients who are already taking antiplatelet agents have reduced platelet function but in some the treatment is not working as effectively as in others. High residual platelet function might have contributed to the stroke or TIA. Platelet function testing via measurement of platelet P-selectin can be performed remotely in the context of a multicentre trial.

Disclosure of Interest: S. Heptinstall Shareholder of: Platelet Solutions Ltd, N. Dovlatova Employee of: Platelet Solutions Ltd, J. May Shareholder of: Platelet Solutions Ltd, K. Robson: None declared, P. Bath Consultant for: Platelet Solutions Ltd.

PO519-TUE

Inhibition of TAFI and PAI-1 is protective in a mouse model of cerebral ischemia/reperfusion injury

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Background: Cerebral ischemia/reperfusion injury is associated with activation of the blood coagulation cascade and fibrin deposition in cerebral microvessels. Endogenous tissue plasminogen activator (tPA)-mediated fibrinolysis is attenuated by thrombin activatable fibrinolysis inhibitor (TAFI) and plasminogen activator inhibitor-1 (PAI-1). Activated TAFI (TAFIa) removes C-terminal lysines on partially degraded fibrin, which impairs efficient plasminogen activation whereas PAI-1 directly inhibits tPA activity.

Aims: To evaluate the therapeutic potential of inhibiting TAFI and PAI-1 in the setting of cerebral ischemia/reperfusion injury.

Methods: C57/Bl6J mice were subjected to 60 min transient middle cerebral artery occlusion. Inhibitory monoclonal antibodies against TAFIa (MA-TCK26D6) and PAI-1 (MA-33H1F7) were administered immediately after the start of reperfusion. Twenty-four hours later, cerebral fibrin(ogen) deposition, infarct size and neurological outcome were assessed.

Results: Administration of either anti-TAFIa (25 mg kg⁻¹) or anti-PAI-1 (6 mg kg⁻¹) monoclonal antibodies significantly decreased cerebral fibrin(ogen) deposition in the ipsilateral cerebral hemisphere ($P < 0.05$), thereby reducing cerebral infarct sizes to $37 \pm 5.6 \text{ mm}^3$ and $36.6 \pm 7.6 \text{ mm}^3$ respectively compared with $80.6 \pm 11.1 \text{ mm}^3$ for control antibody ($P < 0.01$). Concurrently, functional recovery of the

animals was also significantly improved. Interestingly, single targeting of PAI-1 or TAFIa with a lower dose of 1 mg kg⁻¹ inhibiting antibody had no protective effect, but combined targeting of both TAFIa and PAI-1 at the same low dose did reduce cerebral fibrin(ogen) deposition and infarct size by 50% 24 h post reperfusion ($P < 0.05$).

Conclusion: Both combined and single targeting of PAI-1 and TAFIa have a protective effect in a mouse model of cerebral ischemia/reperfusion injury, which could open novel treatment avenues for ischemic stroke.

Disclosure of Interest: None declared.

PO520-TUE

A factor Xa variant improves hemostasis models of intracerebral hemorrhage

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Background: Effective therapies are needed to control excessive bleeding in a range of clinical conditions where bleeding cannot be controlled by topical or surgical intervention. One of the most devastating clinical bleeding settings is intracerebral hemorrhage (ICH), a major cause of death and disability accounting for 10–15% of stroke cases worldwide. FXa^{116L}, a recently described variant of Factor Xa, was designed with an Isoleucine (I) to Leucine substitution at amino acid 16 (based on chymotrypsin numbering). This variant exhibits zymogen-like properties with both reduced activity and sensitivity toward plasma inhibitors. However activity is restored in the presence of its cofactor, Factor Va.

Aims: To study the hemostatic potential of the FXa^{116L} variant in a non-clinical ICH injury models.

Methods: ICH was induced in male CD-1 mice or male Sprague-Dawley rats by injection of bacterial collagenase VII-S into the right striatum of the brain. FXa^{116L} was administered intravenously, at 45 min post-ICH induction in mice and 15 min post-ICH induction in rats. In rats factor eight inhibitor bypass activity (FEIBA) (100 U kg⁻¹) was used as a positive control. The hemorrhagic blood volume was quantified by hemoglobin content using Drabkin's reagent in homogenized whole brain tissue, 24 and 2 h post ICH in mice and rats, respectively.

Results: Compared to vehicle dosed mice, a dose-dependent decrease in the hematoma volume was observed in animals dosed at 1 µg kg⁻¹ (21%), and a maximum response at 200 µg kg⁻¹ (47%) µg kg⁻¹. Rats treated with FXa^{116L} also exhibited a reduced hematoma volume compared to vehicle treated rats; 30 µg kg⁻¹ (27%). In rats dosed with FEIBA the hematoma volume by decreased 17% compared to vehicle treated.

Conclusion: FXa^{116L} decreased the hematoma volume in ICH injury models enhancing hemostasis. Together, these studies suggest that FXa^{116L} may provide a new and unique way to achieve hemostasis in clinical situations of uncontrolled bleeding.

Disclosure of Interest: R. Jasuja Employee of: Pfizer, R. Camire Consultant for: Pfizer, S. Arkin Employee of: Pfizer, D. Pittman Employee of: Pfizer

PO521-TUE

Genetic ablation of extra domain a of cellular fibronectin protects hypercholesterolemic mice from acute stroke by reducing thrombo-inflammation

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Background: Fibronectin containing extra domain A (Fn-EDA) is absent in plasma of healthy humans, but present in several disease states including atherosclerosis.

Aims: We determined the role of Fn-EDA in experimental stroke outcomes in hypercholesterolemic apolipoprotein E-deficient (Apoe^{-/-}) mice, which express Fn-EDA levels in plasma similar to those reported in patients with atherosclerosis.

Methods: Stroke outcome was evaluated in male and female mice using a transient middle cerebral artery occlusion model. Cerebral thrombosis was assessed using Doppler flowmetry. Susceptibility to thrombosis was assessed in a FeCl₃ injury-induced carotid artery thrombosis model.

Results: Irrespective of sex, Fn-EDA^{-/-}/Apoe^{-/-} mice exhibited smaller infarcts and improved neurological outcomes at day 1 and day 7 ($P < 0.01$ vs. Apoe^{-/-}). Concomitantly, postischemic inflammation (neutrophil/macrophage infiltration, phospho-NF-κB p65 and IKKα/β, IL1-β and TNFα protein levels) and cerebral thrombosis within lesions were decreased ($P < 0.01$ vs. Apoe^{-/-}). Using intravital microscopy, we found that the rate of thrombus growth and the time to stable occlusion of the carotid artery were significantly prolonged in Fn-EDA^{-/-}/Apoe^{-/-} mice ($P < 0.01$ vs. Apoe^{-/-}). Genetic deletion of TLR4 protected against stroke and post-ischemic inflammation in Apoe^{-/-} mice ($P < 0.05$ vs. Apoe^{-/-}) but had no effect on stroke outcomes in Fn-EDA^{-/-}/Apoe^{-/-} mice, suggesting that TLR4 contributes to Fn-EDA mediated exacerbates stroke outcome. Bone marrow transplantation experiments revealed that Fn-EDA released from endothelial cells exacerbates stroke outcome through TLR4 expressed on hematopoietic cells. Importantly, infusion of a specific inhibitor of Fn-EDA into Apoe^{-/-} mouse 15 min after reperfusion significantly improved stroke outcome.

Conclusion: Our findings reveal that Fn-EDA exacerbates adverse stroke outcomes by promoting thrombo-inflammation. We suggest that targeting Fn-EDA could be an effective therapeutic strategy in acute ischemic stroke.

Disclosure of Interest: None declared.

PO522-TUE

Effects of the main active component combination between *Astragalus* and *Panax notoginseng* on inflammation and apoptosis of nerve cell after cerebral ischemia-reperfusion

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Background: *Astragalus* and *Panax notoginseng* are used to treat cardio-cerebrovascular diseases in Chinese medicine, often combined together to promote curative effect.

Aims: To probe the effects and mechanisms that Astragaloside IV (AST IV, active component of *Astragalus*) combined with active components of *Panax notoginseng* including Ginsenoside Rg1 (Rg1), Ginsenoside Rb1 (Rb1) and Notoginsenoside R1 (R1) antagonize ischemia/reperfusion (I/R) injury.

Methods: C57BL/6 mice were randomly divided into 8 groups, after being treated for 3 days, cerebral I/R model was established.

Results: All drugs decreased apoptotic rate in Hippocampal CA1, inhibited caspase-3, the effects of the combination were better than of components alone. AST IV, Rg1 down-regulated the level of TNF-α, ICAM-1 mRNA, respectively, R1 reduced both TNF-α and ICAM-1 mRNA. The combination had inhibitory effects on TNF-α, IL-1 β and ICAM-1 mRNA, furthermore, the inhibitions were more than of components alone. AST IV, Rg1, R1 and the combination restrained the

phosphorylation of I κ B α , relieved nuclear translocation rate of NF- κ B, the effects of the combination were greater than of components alone. All drugs suppressed the phosphorylation of JAK1 induced by I/R, meanwhile the expression of p-STAT1 exhibited a decrease in Rg1 and the combination. The decreases of p-JAK1 and p-STAT1 in the combination were more obvious than in components alone. AST IV, Rg1, R1 further augmented GRP78 caused by I/R, R1 attenuated caspase-12, AST IV, Rg1 lessened the phosphorylation of JNK1/2. The combination up-regulated GRP78 while down-regulated caspase-12 and p-JNK1/2, being greater than of components alone.

Conclusion: The main active components combination between *Astragalus* and *Panax notoginseng* can enhance the effects of anti-apoptosis and anti-inflammation on cerebral I/R injury, the mechanisms may be associated with restraining the activation of NF- κ B and JAK1/STAT1 signal pathways, regulating endoplasmic reticulum stress after cerebral ischemia.

Disclosure of Interest: None declared.

PO523-TUE

The angiotensin-converting enzyme gene insertion/deletion polymorphism is associated with increased risk for ischemic stroke but not for myocardial infarction in young Mexican population

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Background: Myocardial infarction (MI) and stroke represent the most important complications of arterial thrombosis disease.

Aims: The aim was to explore the association of insertion/deletion (I/D) polymorphism on the angiotensin-converting enzyme (ACE) gene with MI or stroke in young Mexican population.

Methods: In a first case-control study 297 patients < 45 years old with MI were included. The diagnosis of MI was based on an electrocardiogram, clinical data and laboratory. They were admitted to the ICCU of the Cardiology Hospital, CMN Siglo XXI, in Mexico City. A total of 297 subjects without history of MI, age-gender matched, were included in the control group. In a second case-control study, 235 patients < 45 years old with stroke were enrolled. Diagnosis of ischemic stroke was considered in all patients after an acute focal neurological deficit with duration > 24 h and followed by confirmation by brain-computed tomography or magnetic resonance. A total of 235 subjects without history of stroke, age-gender matched, were included in the control group. Informed written consent was obtained from all subjects before enrollment.

Results: There was significant difference in the I/D genotype distribution ($P = 0.02$) and allele frequency (OR=1.45, 95% CI 1.0–2.74, $P = 0.02$), between ischemic stroke and control groups. In contrast, there was a similar I/D genotype distribution ($P = 0.65$) and allele frequency ($P = 0.67$), between MI and control groups.

Conclusion: The ID polymorphism of the ACE gene represented an independent risk factor for ischemic stroke but not for MI in young Mexican individuals. Our results suggest a possible different role of genetics factors on atherothrombotic disease such as MI and stroke. The identification of 'at risk' individuals by genetic mapping of susceptible genes for effective control of other host factors will be a effective and practical approach for prevention, as well as the development of therapy for patients.

Disclosure of Interest: None declared.

PO524-TUE

Thromboelastometry (ROTEM®) profiles in patients undergoing thrombolytic therapy for acute ischemic stroke

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Background: Intracerebral haemorrhage remains one of the most feared complications of thrombolytic therapy after an acute ischemic stroke. Standard coagulation monitoring before and after thrombolysis is not able to predict which patients are at a higher risk of bleeding after thrombolysis.

Aims: To evaluate the role of whole blood thromboelastometry, performed by ROTEM®, in predicting bleeding complications in patients with acute ischemic stroke undergoing thrombolytic therapy.

Methods: Twenty-five patients (M/F 15/10, age range 65 ± 16 years) with an acute ischemic stroke who were eligible for recombinant human tissue plasminogen activator (rt-PA) therapy were enrolled. All patients gave informed consent. ROTEM® profiles were obtained just before and 60 min after 0.9 mg kg⁻¹ rt-PA administration, and mean values before and after rt-PA administration were compared.

Results: Of the 25 patients, five (20%) experienced a hemorrhagic event after thrombolytic therapy. A significant reduction of mean basal Maximum Clot Firmness (MCF) in INTEM, EXTEM and FIBTEM tests was observed ($P = 0.035$, 0.033 and 0.002, respectively) after rt-PA treatment. Interestingly enough, the group of patients who developed bleeding complications after rt-PA treatment showed significantly lower mean basal MCF values in FIBTEM assay (13 ± 4 mm) than patients without bleeding events (21 ± 5 mm, $P < 0.006$). Moreover, the five patients who developed bleeding complications showed significantly higher mean Maximum Lysis (ML) values in the EXTEM assay ($14 \pm 3\%$) after rt-PA treatment than patients without bleeding events ($7 \pm 4\%$, $P = 0.007$). ROC curves, performed to measure the diagnostic accuracy of MCF, showed high sensitivity (94.4%) for MCF levels < 13.5 mm in predicting bleeding events, but very low specificity (60%).

Conclusion: Whole blood thromboelastometry could be a useful tool to identify those patients, scheduled for thrombolytic therapy after acute ischemic stroke, at higher risk of bleeding, determining different management strategies after thrombolysis.

Disclosure of Interest: None declared.

PO525-TUE

Severe obesity is a risk factor for cerebral venous thrombosis: a case-control study

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Background: Obesity is a risk factor for deep vein thrombosis of the leg. Whether this is also the case for cerebral venous thrombosis (CVT) is unknown.

Aims: To assess if obesity is a risk factor for CVT.

Methods: Unmatched case control study. For cases we used patients with CVT included in a prospective study between 2006 and 2014 in 2 academic hospitals in The Netherlands and Switzerland. Healthy sub-

jects included in the Dutch MEGA study (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis) were used as controls. Women who were pregnant or had recently given birth were excluded. WHO definitions for overweight (body mass index [BMI] $\geq 25 \text{ kg m}^{-2}$) and obesity (BMI $\geq 30 \text{ kg m}^{-2}$) were used. Using logistic regression analysis we adjusted for sex, age, malignancy, ethnicity, smoking, and oral contraceptive use. A BMI of 18.5–24.99 was used as reference category.

Results: We included 113 cases and 5611 controls. Cases were younger (median 40 vs. 49 years), more often female (75 vs. 53%) and more often used oral contraceptives (72 vs. 23% of women) than controls. Mean BMI was higher in cases compared to controls (26.8 vs. 25.6, $P = 0.05$). Severe obesity was associated with CVT in a dose-dependent manner (BMI 30–34.99 adjusted OR 1.2, 95% CI 0.6–2.5; BMI 35–39.99 adjusted OR 4.5, 95% CI 2.0–10.4; BMI ≥ 40 adjusted OR 9.7, 95% CI 3.2–29.3). When included as a continuous variable, BMI was also associated with CVT (adjusted OR per 1 kg m^{-2} increase in BMI 1.1, 95% CI 1.04–1.1).

Conclusion: Severe obesity is a risk factor for CVT in a dose-dependent manner.

Disclosure of Interest: None declared.

PO526-TUE

Light-sheet fluorescence microscopy of the acutely ischemic brain reveals dramatically reduced thrombotic activity in t-cell deficient mice

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Background: Stroke is the second leading cause of death and disability worldwide and mostly caused by thromboembolic occlusion of major brain vessels. The primary therapeutic goal in acute stroke is to achieve recanalization, but, even if successful, only less than one third of patients benefit. The phenomenon of ongoing ischemic lesion development despite recanalization is referred to as *reperfusion injury*. Although numerous contributing factors, like platelet adhesion, degranulation, as well as T-cell responses have been identified, the mechanistic interactions of these factors leading to brain injury remain unknown and their elucidation requires novel *in vivo* imaging technologies.

Aims: We sought to analyze the spatio-temporal characteristics of platelet deposition and thrombus formation in the ischemic brain using light-sheet fluorescence microscopy (LSFM) in wildtype and T-cell deficient mice (*Rag1*^{-/-}).

Methods: We subjected mice to transient middle cerebral artery occlusion (tMCAO), a model of ischemic stroke. Brains were antibody-stained for platelets and endothelial cells, paraformaldehyde-fixed and chemically cleared. Entire hemispheres were imaged via LSFM and thrombus formation was mapped.

Results: We established a protocol for antibody penetration, tissue clearing, and triple-color illumination allowing the visualization of platelet deposition in an intact brain hemisphere. This approach allowed us to generate a spatio-temporal map of thrombotic activity in the microcirculation of the ischemic brain, revealing the gradual increase of formed thrombi, while an unexpectedly high number of brain vessels remained perfused. Strikingly, T-cell deficient mice displayed dramatically reduced numbers of thrombi in the ischemic brain, despite normal thrombus formation in models of arterial thrombosis.

Conclusion: This novel method allows a spatio-temporal mapping of cell-cell interactions during cerebral infarct progression, thereby

revealing fundamental pathomechanisms of thrombo-inflammation in the ischemic brain.

Disclosure of Interest: None declared.

PO527-TUE

The alteration of iron transmembrane proteins expression in the rat hippocampus following focal cerebral ischemia

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Background: Iron is an important micronutrient for neuronal function and survival. It plays an essential role in synthesis of the myelin sheath and neurotransmission. On the contrary, iron also catalyses the production of free radicals and hence, causes oxidative stress. Therefore, maintenance of iron homeostasis is very crucial and it involves a number of proteins in iron metabolism and transport that maintain the balance. Iron distribution and expression of iron transmembrane protein in cerebral ischemia are unknown.

Aims: In this study, we focus on the changes of iron transmembrane protein expression, such as Transferrin Receptor (TFR), Ferroportin (Fpn), Feline leukemia virus subgroup C receptor (FLVCR) and Breast cancer resistance protein (BCRP), in the rat hippocampus following focal cerebral ischemia.

Methods: Rats were randomly divided into 2, 6, 12, 24 and 72 h groups following middle cerebral artery occlusion (MCAO) and the mRNA and protein level of iron transmembrane proteins were detected by reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry (IHC) at the above time points respectively.

Results: The expression of TFR was significantly increased in the hippocampus at 12 h after surgery and maintained in a high level at 24 h and 72 h, whereas BCRP was reduced at 12 h after surgery and maintained at low level at 24 h and 72 h. The expression level of FLVCR was significantly decreased at 72 h, while the expression of Fpn was increased, which is reached a peak at 12 h after surgery.

Conclusion: Our findings strongly suggest that dysregulation of intracellular iron balance is possibly a new mechanism underlying cerebral ischemia and that targeting iron homeostasis may serve as a potential therapeutic approach to combat cerebral ischemia.

Disclosure of Interest: None declared.

PO528-TUE

Nonclinical and clinical development of TMS-007, a member of the anti-inflammatory small-molecule thrombolytic SMTPs

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Background: SMTPs, a family of small molecules derived from a fungus, modulate plasminogen conformation and enhance its proteolytic activation, thus promoting physiological plasmin formation and thereby effectively treat thrombotic and embolic strokes in animal models in rodents and primates. Notably, the SMTP compound reduces hemorrhagic transformation and has a wide therapeutic time

window, both of which are attributable to the combination of thrombolysis with anti-inflammation via inhibition of soluble epoxide hydrolase, a key enzyme involved in the regulation of the levels of anti-inflammatory epoxy-fatty acids.

Aims: The goal of this project is to develop a SMTP congener as a drug widely prescribable to stroke patients.

Methods: The standard nonclinical testings were performed according to the ICH guidelines using a GMP grade TMS-007, one of the promising SMTP compounds, of which manufacturing methods, specifications, and testing methods were established. Pharmacological studies were performed with various ischemic stroke models in rodents and primates. A randomized, placebo-controlled, double blind phase I study of TMS-007 was performed with healthy male volunteers aged from 20 to 45 years. The study consisted of 6 cohorts differing in the dose from 3 to 600 mg per body by a single intravenous infusion for 30 min.

Results: The GMP production of TMS-007 was accomplished by fermentation, followed by several purification steps, affording highly purified API. Both API and the injection formulation were stable. In embolic stroke models in the mouse, gerbil, and monkey, TMS-007 was efficacious in reducing infarct size and ameliorating neurological deficits with treatment 3–6 h after embolization. In atherothrombotic stroke model in monkeys, TMS-007 suppressed infarct development and hemorrhagic transformation. No obvious toxicity was observed at a pharmacological dose of TMS-007 in the GLP testing. The phase I study is ongoing.

Conclusion: TMS-007 is a promising candidate of a new generation stroke drug.

Disclosure of Interest: None declared.

PO529-TUE

Antithrombin levels in adult stroke patients at the university of Benin teaching hospital

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Background: Antithrombin deficiency has been postulated to be associated with ischaemic stroke. ischaemic stroke is relatively common in Nigeria. The role of antithrombin deficiency in the aetiopathogenesis of ischaemic stroke has not been fully determined in Nigerians with ischaemic stroke

Aims: To determine the role of antithrombin deficiency in the development of ischaemic stroke in Nigerians

Methods: One hundred and thirty (130) participants comprising 65 ischaemic stroke patients and 65 apparently healthy control. Functional and quantitative assessment of antithrombin was done using chromogenic assay and ELISA methods from Technochrom AT111 kit and Assaypro ELISA kit respectively. The PT and APTT were done using standard methods

Results: A total of 65 patients with ischaemic stroke mean age 60.4 ± 12.3 years were studied. Total WBC was significantly higher in stroke subjects than controls ($P = 0.001$). The mean platelet count was also higher in ischaemic stroke subjects than healthy controls. the Hb concentration was lower in ischaemic stroke subjects. The PT and APTT were similar in both groups. There was no statistically significant difference between plasma antithrombin level in subjects with stroke and controls

Conclusion: Antithrombin deficiency may not be associated with the pathogenesis of ischaemic stroke in Nigerians. larger population studies are needed to determine the exact role of antithrombin in Nigerians with Ischaemic stroke.

Disclosure of Interest: None declared.

PO530-TUE

Clopidogrel resistance after minor ischemic stroke or TIA is associated to pre-diabetes, hypertension and cerebral microangiopathy

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Background: Clopidogrel resistance (CR) or 'high on-treatment platelet reactivity' confers an increased risk of recurrent CVD and cardiovascular death.

Aims: Investigate the importance of glucose metabolism, hypertension and micro- and macrovascular pathology for CR in patients with minor ischemic stroke (IS) or TIA.

Methods: Seventy-two patients treated with clopidogrel were evaluated 1 month after symptoms. Platelet function was measured by whole-blood impedance aggregometry and CR defined according to recent consensus. Glucose metabolism was evaluated by oral glucose tolerance test (OGTT) and fasting serum insulin (fS-insulin). Cerebral microangiopathy was evaluated by Fazekas scale for white matter changes (WMC) on CT brain. Carotid atherosclerosis was evaluated by ultrasound or CT/MR angiography. Complementary sampling for microalbuminuria and hsCRP was performed in the stable phase for a subset of patients.

Results: CR was found in 22% (16/72) of patients. CR had a more disturbed glucose metabolism than responders (R), with median fasting plasma glucose 5.9 vs. 5.2 mmol L⁻¹ ($P = 0.02$), prevalence of pathological OGTT/DM 88% vs. 46% ($P = 0.002$), and fS-insulin 99 vs. 57 pmol L⁻¹ ($P = 0.03$) compared to R. HbA_{1c} did not differ between groups. CR received median two antihypertensive drugs vs. one for R, and had higher systolic blood pressure, 145 vs. 135 mm Hg ($P = 0.02$). Presence of moderate to extensive cerebral WMC was more common in CR than R, 56% vs. 25% ($P = 0.02$). There were no differences between groups in degree of carotid atherosclerosis. Microalbuminuria or hsCRP did not differ between 11/16 CR and 48/56 R sampled more than 1 year after symptoms.

Conclusion: Pre-diabetes, hypertension and cerebral microangiopathy are associated with CR after minor IS or TIA, but not carotid atherosclerosis, nephropathy or chronic systemic inflammation. Endothelial dysfunction and shear activation of platelets in the cerebral microvasculature may contribute to the results.

Disclosure of Interest: None declared.

PO531-TUE

Association between polymorphisms in microRNA machinery genes (DICER1, DROSHA, RAN, AND XPO5) and ischemic stroke

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Background: Stroke is the third leading cause of death in the world. Ischemic stroke is a complex, multifactorial disease influenced by multiple genetic and environmental factors. MicroRNAs (miRNAs) play a role in atherosclerosis-related diseases, such as cerebrovascular and cardiovascular disease. In addition, miRNA machinery genes, such as

DICER, DROSHA, RAN and XPO5, play an important role in vascular disease

Aims: The objective of this study was to investigate the association between ischemic stroke and six known polymorphisms in miRNA processing genes [DICER1 rs13078 3' untranslated region (UTR) A>T, DICER1 rs3742330 3'UTR A>G, DROSHA rs10719 3'UTR T>C, DROSHA rs6877842 3'UTR G>C, RAN rs14035 3'UTR C>T, and XPO5 rs11077 3'UTR A>C] with ischemic stroke in Koreans. A total of 1000 participants (589 ischemic stroke patients and 411 controls) were enrolled in the study

Methods: We analyzed the associations between these polymorphisms and both disease status and clinical factors in 585 ischemic stroke patients and 403 controls. Genotyping was performed with the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method

Results: We identified an association between the DICER1 rs3742330 and DROSHA rs10719 polymorphisms and ischemic stroke. The frequency of the rs3742330 and rs10719 polymorphisms was higher in stroke patients than in controls, suggesting these polymorphisms may be associated with increased risk of ischemic stroke

Conclusion: Our study provides the evidences for DICER and DROSHA genes as potential biomarkers for use in ischemic stroke prevalence

Disclosure of Interest: None declared.

PO532-TUE

Thrombolysis using N-Acetylcysteine in acute ischemic stroke

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Background: The recanalization rate after tPA-induced fibrinolysis remains low (~30%), especially in case of platelet-rich thrombi (~6%). We recently demonstrated that platelet cross-linking during high-shear thrombosis in intracranial arteries is mediated by von Willebrand Factor (VWF) polymers. Interestingly, since VWF polymerization involves disulfide bonds, thiol reducing agents (including N-Acetylcysteine, NAC) are able to cleave VWF multimers.

Aims: (i) To investigate whether NAC would disperse platelet aggregates by reducing VWF multimers and (ii) to compare this thrombolytic strategy to fibrinolysis in terms of recanalization rate in different murine models of ischemic stroke.

Methods: Thrombin and FeCl₃ induced stroke in mice.

Results: We first compared two thrombotic stroke models induced either by intra-arterial administration of thrombin or by topical application of ferric chloride (FeCl₃) in terms of clot composition and sensitivity to tPA. Whereas thrombin induced fibrin-rich clots, sensitive to tPA, FeCl₃ induced the formation of platelet-rich clots which were resistant. Accordingly, tPA failed to improve stroke outcome in the FeCl₃ model. In contrast, a single intravenous administration of NAC (400 mg kg⁻¹) restored vessel patency and improved stroke outcome in this model. This thrombolytic effect was further potentiated by co-administration of NAC with a GpIIb/IIIa inhibitor, leading to a 77% reduction in ischemic lesion size when injected 20 min after stroke onset. Interestingly, in contrast to heparin, NAC did not worsen intracranial hemorrhage.

Conclusion: NAC is superior to tPA for disaggregating platelet-rich thrombi in mice and does not promote hemorrhagic transformations in our experimental conditions. Therefore, thiol-reducing agents represent a new class of thrombolytics.

Disclosure of Interest: None declared.

PO533-TUE

Multiple meningiomas does not increase the risk of intracranial hemorrhage after intravenous thrombolysis for acute ischemic stroke

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Background: Intravenous Thrombolysis is the only FDA approved pharmacological therapy for acute ischemic stroke. Intracranial neoplasm are currently considered as a contraindication for IV thrombolysis in acute ischemic stroke patients.

Aims: We report a case of acute ischemic stroke that received IV thrombolysis within 3 h of symptom onset and was found to have 2 meningiomas on subsequent brain imaging.

Methods: An 83-year-old woman developed sudden onset right-sided hemiparesis and aphasia. She presented with disabling neurological deficit with a NIHSS of 19. Initial head CT at another hospital was read as 'no acute abnormality'. We only had access to the report of the initial head CT scan and were not able to review the images. She had no obvious contraindication for IV rtPA. She received IV rtPA based on the standard protocol and afterwards, she was transferred to our medical center for higher level of care. On presentation to our ER, 6 h after the symptom onset, she had improvement in her language function and her NIHSS was 14. A non-contrast CT head was done right after, which showed no ICH but showed a 1.4 cm and a 2.2 mm hyperdense mass suggestive of a planum sphenoidale meningioma. Brain MRI with and without gadolinium was performed 6 h later which demonstrated acute ischemic infarct in the left frontal lobe. In addition, brain MRI revealed 2 meningiomas. Head CT scan was repeated in 24 h from IV rtPA administration that did not show ICH. Over next 48 h NIHSS improved to 9, and to 4 upon discharge on day 4 post IV rtPA administration.

Results: To the best of our knowledge, this is the first reported patient of acute ischemic stroke who received IV thrombolysis and had more than one intracranial neoplasms, both were meningiomas. Our case confirms the previous literature that the presence of benign intracranial neoplasms does not increase the risk of ICH in acute ischemic stroke patients who receive IV thrombolysis.

Conclusion: NA.

Disclosure of Interest: None declared.

PO534-TUE

Patent foramen ovale in patients with previous cerebral ischemic events: echocardiographic features and relationships with transcranial Doppler

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Background: The association of patent foramen ovale (PFO) with stroke has been repeatedly confirmed. PFO is found frequently in patients with 'cryptogenic' stroke (i.e. not attributable to traditional cardiovascular risk factors).

Aims: We assessed the echocardiographic features and their relations with transcranial Doppler in patients with PFO and history of cryptogenic cerebral ischemic events.

Methods: The study population included 97 consecutive outpatients with a recent cerebral ischemic event, referred for PFO search from January 2011 to December 2013. All patients underwent a complete transthoracic echocardiography (TTE), with additional bubble test of injected saline solution (to visualize transition of bubbles from right to left atrium) and transcranial Doppler (TCD) bubble test of middle cerebral artery (to visualize high-intensity transient signals [HITS] at the cerebral level). Patients were divided into 2 groups: 65 with PFO and 32 without PFO.

Results: The two groups were comparable for sex, age, body mass index, blood pressure and heart rate. Patients with positive 'bubble' TTE exhibited larger right atrial (RA) diameter (3.6 ± 0.6 cm vs. 3.4 ± 0.5 cm, $P < 0.01$) than patients without PFO. In patients with PFO, RA diameter normalized for body surface area was greater in PFO patients with TTE derived microbubbles > 20 than in those with < 20 microbubbles. It was also related with HITS number, calculated in the cycle with the highest number by TCD (β coefficient = 0.325, $P < 0.005$), independent of potential confounders (Chiari network, atrial septal aneurism and redundancy of Eustachian valve).

Conclusion: PFO is associated with enlarged right atrium size. RA enlargement is an independent correlate of HITS number in the cerebral district. We suggest that RA size should be studied as a potential additional risk factor for PFO-related stroke.

Disclosure of Interest: None declared.

PO535-TUE

Hemorheology and hemostasis in ischemic stroke patients treated with i.v. thrombolysis

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Background: Hemostatic and hemorheological disturbances are the typical pathological conditions in ischemic stroke and may be related with microcirculatory failure (MCF) suspected to be responsible for the non-reperfusion after recanalization therapy.

Aims: To evaluate the association of hemostatic and hemorheological features with clinical parameters and neuroimaging correlates of MCF in stroke patients treated with or without i.v. thrombolysis (IVT).

Methods: Detail dynamic study of hemostasis (fibrinogen, INR, D-dimer, PTT, APTT), platelet aggregation on ADF and on adrenaline, kinetics of erythrocyte aggregation/disaggregation and erythrocyte deformability (light-backscattering techniques) in comparison with clinical (NIHSS) and neuroimaging features (MRI, MR-angiography, CT-perfusion) and functional outcome (mRS) were performed in ischemic stroke patients treated with IVT (group 1) or without it (group 2). All participants or their representative had signed informed consent.

Results: The positive association of fibrinogen level at admission with NIHSS after IVT was found in atherothrombotic stroke (ATS) ($r = 0.673$, $P = 0.023$). The activity of platelet aggregation on adrenaline (PA-Adr, %) was initially increased in group 1 and in patients with ATS. The rate of its diminution was higher in group 1. Neurological improvement was more rapid in group 1. Increased strength of erythrocyte aggregates (SEA) was found to be associated with better functional outcome (mRS) and that correlation was stronger in group 1 (for group 1 $r = -0.703$, $P = 0.016$; for group 2 $r = -0.531$, $P = 0.034$). Increased SEA was also associated with better dynamics of ischemic lesion on DWI-MRI on 3 day in group 2.

Conclusion: Activation of PA-Adr and association of fibrinogen with NIHSS reflects predominantly platelet dysfunction during ATS. Systemic action of IVT may cause reduction of PA-Adr in group 1.

Increased SEA which may be assumed as compensatory reaction seems to be a prognostic for better outcome in stroke patients.

Disclosure of Interest: None declared.

PO536-TUE

Altered fibrin clot structure/function in patients with cerebral sinus venous thrombosis and the risk of recurrence

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Background: Venous thromboembolism is associated with a prothrombotic fibrin clot phenotype.

Aims: We tested the hypothesis that denser fibrin networks displaying impaired lysisability characterize patients with cerebral sinus venous thrombosis (CSVT).

Methods: We assessed the ex vivo plasma fibrin clot properties, including lysisability, in 50 patients following the first CSVT after withdrawal of anticoagulation (aged 38.9 ± 9.8 years, female 72%) and 50 controls matched for age and sex. Patients with trauma or cancer were ineligible. Recurrences were recorded during a 3-year follow-up.

Results: The CVST and control groups differed with regard to FV Leiden (22 vs. 6%, $P = 0.04$) but not in terms of other CVST risk factors. D-dimer, fibrinogen and plasminogen activator inhibitor-1 were similar in both groups. Clot permeability (K_s) was lower in CSVT patients than in controls (6.43 ± 0.97 vs. $7.3 \pm 1.2 \cdot 10^{-9}$ cm², $P < 0.001$) and was associated with longer clot lysis time induced by tissue plasminogen activator (103.0 ± 16.8 vs. 92.4 ± 16.2 min, $P = 0.002$) as well as with lower maximum rate of D-dimer release from a clot (0.068 [0.064–0.071] vs. 0.072 [0.067–0.078] mg L⁻¹ min⁻¹, $P = 0.0015$), respectively. CSVT patients had a slightly shorter lag phase (40 [37–42] vs. 41 [38–44] s, $P = 0.039$) and higher maximum absorbance of fibrin gel (0.88 [0.81–0.92] vs. 0.80 [0.76–0.86], $P < 0.001$) compared to controls. Fibrin clot variables were not associated with provoked/unprovoked event or thrombophilia. During 3 years following the first event, CVST recurred in six patients (12%), who had higher levels of fibrinogen (3.36 [3.17–3.95] vs. 2.91 [2.65–3.21] g L⁻¹, $P = 0.006$). Regression analysis showed that after adjustment for fibrinogen CVST recurrence was independently associated with K_s (OR, 7.1; 95% CI, 1.7–29.9; lower quartile vs. the remainder) and lag phase (OR, 5.9; 95% CI, 1.2–29.9).

Conclusion: This study is the first to show that CSVT is associated with altered fibrin clot structure and resistance to fibrinolysis, which may predispose to its recurrence.

Disclosure of Interest: None declared.

PO537-TUE

The novel biased agonist for PAR-1 protects brain cells from toxicity similar to activated protein C

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Background: Activated protein C (APC) is a homeostatic serine protease with antithrombotic activities and also cytoprotective actions, which realizes via PAR-1 and endothelial protein C receptor. Thrombin interacts with PAR-1 also, but demonstrates effects opposite to APC's actions during excitotoxicity and inflammation. In contrast to thrombin APC protects cells from damage at stress, hypoxia and toxic

conditions. It has been shown recently that the multidirectional effects of thrombin and APC may be due to biased agonism at the action of thrombin and APC proteinases on endothelial cell PAR-1 (Mosnier et al., 2012).

Aims: The goal of this work was to study the influence of peptide analog of the PAR-1 tethered ligand liberated by APC (AP9) on rat astrocyte and neuron cultures at toxic conditions.

Methods: We have used following models: 1 - neuroinflammation; 2 - glutamate excitotoxicity. 1. It was performed on astrocytes cultures with using as inflammation agent high concentration of thrombin (50 nM). 2. Cell survival was measured 24 h after 30-min incubation neuronal rat cultures with 100 μ M glutamate. The cell proliferation of primary cortical astrocyte culture was estimated by MTT method. The peptide NPNDKYEPF-NH₂ (AP9) was synthesized in Russian cardiology research and production complex.

Results: We shown that AP9 (0.2–200 μ M) in dose-dependent manner decreases the thrombin-induced activation of astrocytes like APC. AP9 prevents proliferation and increasing of S100B level and normalizes cytoskeleton of astrocytes at thrombin toxicity. The results of this research demonstrate for the first time the neuroprotective effect of AP9 at excitotoxicity similar to the effects of APC. The blocking of PAR-1 with SCH79797 prevents AP9 effects on astrocytes as well as on neurons.

Conclusion: Thus AP9 has neuroprotective effects and prevents the activation of astrocytes and astrogliosis during pathological condition similar to APC via PAR-1.

Disclosure of Interest: None declared.

Thrombophilia II

PO538-TUE

Role of genetic polymorphisms of haemostasis's system in case of habitual miscarriage

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Background: The problem of habitual miscarriage is one of the most difficult medical and social problems. Currently there are no data can be taken from Russian sources on the epidemiology of genetic predisposition to thrombophilia among women with habitual miscarriage at the European North of Russia. But it's known that the north region takes the first places in spontaneous abortion level.

Aims: To obtain data on the epidemiology of some genetic polymorphisms among women with complicated obstetrical history in the European North of Russia.

Methods: A retrospective research of the genetic polymorphisms epidemiology was conducted. Target group consists of 185 women with recurrent miscarriage and no thrombotic episodes. As a control group, we used data on the prevalence of the same genetic polymorphisms among women in Arkhangelsk, who had no history of thrombotic episodes ($n = 74$). Further analysis was performed for the presence of seven genetic polymorphisms that determine defects of hemostasis and predisposing to thrombosis: fibrinogen G455A, prothrombin G20210A, clotting factor V G1691A, MTHFR C677T, PAI-I 675 4G/5G, platelet receptors GpIIIa T1565C, ApoE E2/E3/E4.

Results: Despite the absence of statistically significant differences between groups, it is important to note a fairly high odds ratio for gene PAI-I 675 4G/4G (OR = 1.80; 95% CI 0.98–3.31; $P = 0.058$), gene GpIIIa 565 T/C (OR=1.74; 95% CI 0.95–3.10; $P = 0.074$) and the gene ApoE E2/E4 (OR=4.22; 95% CI 0.76–23.42; $P = 0.092$).

Conclusion: All three polymorphisms are potential risk factors for thrombosis.

Disclosure of Interest: None declared.

PO539-TUE

Individual absolute and relative risk (RR) of pulmonary embolism (PE) in pregnancy and puerperium in presence of hereditary thrombophilic risk factors

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Background: PE is a major cause of maternal death.

Aims: The aim of our study was to determine the individual probability of PE based on the relative and absolute risk associated with hereditary risk factors of thrombosis. These data have not been available to date.

Methods: In 243 women with first venous thromboembolism (VTE) in pregnancy and 243 age-matched women with at least one prior pregnancy and no history of VTE, RR for hereditary thrombophilic risk factors and the individual probability of PE were determined. Based on epidemiological data, an incidence of approx. 1:3500 deliveries was assumed.

Results: RR of VTE was 4.6 for heterozygous factor V Leiden (FVL), 20 for homozygous FVL, 3.1 for heterozygous prothrombin gene mutation (PTM), 44 (estimated) for combined heterozygous FVL and PTM, 2.0 ($P = 0.083$) for antithrombin (AT) < 90% (10th percentile) and 49 for < 60%, 2.3 for protein C deficiency < 76% (10th percentile) and 5.5 for < 50%, 4.1 for protein S deficiency < 40% ($P = 0.089$), and 9.7 for free proteinS deficiency < 40% ($P < 0.05$ for all unless noted otherwise). Probability of pregnancy-related PE was 0.1% for heterozygous FVL, 0.57% for homozygous FVL, 0.08% for heterozygous PTM, 1.2% for combined heterozygous FVL and heterozygous PTM, 0.06% for AT deficiency < 90%, and 1.4% for < 60%, 0.06% for protein C deficiency < 76%, 0.16% for < 50%, 0.10% for protein S deficiency < 40%, and 0.29% for free protein S deficiency < 40%. FVL-associated risk may be lower (FVL paradox).

Conclusion: This study presents the first data available for pregnancy-related PE associated with thrombophilic risk factors. A relevant increase in the risk of PE was estimated in patients with severe thrombophilia (e.g. combined defects and AT deficiency). Since 7 of 17 women with VTE and homozygous or combined heterozygous FVL/PTM had no first-degree relatives with a history of VTE, current guidelines recommending to omit heparin prophylaxis in these patients are to be questioned, especially considering the assumed case fatality rate of 10%.

Disclosure of Interest: None declared.

PO540-TUE

R147W in proc gene and the risk of thromboembolism in Thai children

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Background: Protein C (PC) deficiency is a common risk factor of thromboembolism (TE) in Asian population. The incidence of PC deficiency in Thai population and patients with venous TE were 1.99% and 8.9%, respectively, which were higher than in Caucasians. R147W mutation, an nt6152 C>T in exon 7, has been reported to be a common

mutation in Chinese population with venous TE. However, this mutation has never been studied in Thai children.

Aims: To determine the risk factor of R147W mutation in Thai children with TE.

Methods: Patients age < 19 years diagnosed with TE were informed to be enrolled in the study. The controls were healthy regular donors without history of TE from hospital Blood Bank. PC gene was amplified by polymerase chain reaction using specific primer in exon 7.

Results: A total of 190 patients and 485 controls, with mean ages of 8.6 years and 33.1 years, respectively, were enrolled. The diagnosis of TE was arterial ischemic stroke (AIS) 52.1%, venous TE 21.0%, systemic arterial TE 13.1%, cerebral venous sinus thrombosis (CVST) 12.1%, and multiple sites TE 1.6%. The underlying diseases, identified in 52.6% of patients, were vasculitis (29%) followed by malignancy (14%) and thalassemia diseases (12%). The incidence of heterozygous and homozygous R147W in patients and controls was 9.5% vs. 5.8% and 2.6% vs. 0.0%, respectively. Heterozygous R147W had odds ratios of 3.0 (95% CI 1.2–7.9, $P = 0.016$) and 4.5 (95%CI 1.6–13.1, $P = 0.002$) to develop venous TE and CVST, respectively. In addition, heterozygous R147W in patients without underlying diseases had OR of 2.1 (95%CI 1.0–4.5, $P = 0.048$) for developing TE. PC activity in patients with normal, heterozygous and homozygous R147W had the mean (SD) levels of 113.0 (45.5), 63.5 (21.8) and 58.0 (39.0) %, respectively, $P < 0.001$.

Conclusion: R147W is a common mutation in Thai children with TE. Therefore, R147W should be included as a screening test in children with venous TE, CVST and TE without underlying diseases.

Disclosure of Interest: None declared.

PO541-TUE

Acute superior mesenteric vein thrombosis in a non-cirrhotic patient with heterozygous G20210A prothrombin gene mutation: a case report

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Background: A 40-year-old man presented with acute onset abdominal pain and nausea. Exam revealed diffuse abdominal tenderness. CBC, electrolytes and liver profile were unremarkable. Abdominal CT demonstrated acute thrombus in the superior mesenteric vein, without liver cirrhosis. Anticoagulation was initiated. Imaging and colonoscopy, autoimmune panel and genetic testing for JAK2 mutation were negative. Thrombophilia studies were only positive for heterozygous form of prothrombin 20210G>A mutation. Follow-up CT scan at 5 months showed complete clot resolution.

Aims: Report a case of splanchnic venous thrombosis in non-cirrhotic patient, discuss clinical presentation, work-up, and management of this entity and implications of thrombophilia

Methods: Per Mr. Celine from ISTH 2015 Abs [isth2015abs@mci-group.com], I can submit case report here.

Results: Splanchnic vein thrombosis (SVT) unrelated to cirrhosis is rare and potentially lethal. Clinical presentations include vague GI symptoms. Abdominal CT is the diagnostic modality of choice. Anticoagulation should be initiated promptly to prevent extension of thrombus and intestinal infarction. Work-up should include evaluation of local risk factors, such as liver cirrhosis, infections or malignancies, as well as thrombophilia screening to rule out inherited hypercoagulable states. Expert consensus recommends anticoagulation for at least 3 months for non-cirrhotic SVT. Lifelong anticoagulation is recommended in cases of underlying prothrombotic factors. Heterozygous mutation of prothrombin gene carries a 2.8 fold increased risk of thrombosis; however, unlike those with homozygous mutation, guidelines recommend same anticoagulation duration as with normal genotype.

Conclusion: Non-Cirrhotic SVT is rare but potentially lethal condition that warrants prompt recognition. Anticoagulation should be started promptly and evaluation should include thrombophilia screening, as it may determine optimal duration of treatment.

Disclosure of Interest: None declared.

PO542-TUE

Thrombin generation test at R2 polymorphism of factor V

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Background: Thrombin generation test (TGT) is a functional test with a potential to assess the capacity of complete hemostatic system unlike routinely used coagulation tests that reflect specific part of coagulation system only. TGT has a special area of application in the determination of real prothrombotic effect of known thrombophilias. R2 polymorphism of factor V (FV A4070G) increases the risk of thrombotic disease only slightly according to recent experience.

Aims: The aim of our paper was to assess the prothrombotic effect of R2 FV using TGT test.

Methods: We performed thrombin generation test in the cohort of R2 FV heterozygotes and in a control group. All the assessed individuals had negative personal history of thrombotic complications. Thrombin generation test: Venous blood was collected into 0.1 volume of 3.6% trisodium citrate, centrifuged for 20 min at 2000 g and stored at -80°C until time of analysis. Three major parameters of thrombin generation curve were used for analysis – maximum of thrombin generation (TGT max), endogenous thrombin potential (TGT ETP, area under curve) and TGT lag time. R2 polymorphism FV was detected by allele specific PCR.

Results: The group of patients with R2 polymorphism of FV had thrombin generation with significantly higher value only of TGT max 229, 4 (nM), but not ETP 2175 (nM) and lag time 3.0 (min) in comparison with control group TGT max 123, 5 (nM); ETP 2380, 7 (nM); lag time 3.6 (min). The total value of thrombin generation was not significantly different between both groups.

Conclusion: Our results show increased formation and a higher peak of formed thrombin, whereas the total level of thrombin using ETP parameter was without any significant difference in the assessed groups. Our results support clinical experience of just a moderate increase of the risk of thrombotic disease in R2 FV polymorphism heterozygotes.

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PO543-TUE

Factor V Leiden in the absence of APC resistance in a patient after liver transplantation

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Background: Mutation c.1601G>A (p.Arg534Gln) in coagulation factor V gene (F5) makes the molecule less sensitive to proteolytic inactivation by activated protein C (APC) and such phenomenon is known as APC resistance (APCr). This genetic defect (factor V Leiden, FVL) is the most common cause of inherited thrombophilia in European populations. FVL accounts for over 90% of APCr cases, therefore detection of FVL in a patient without APCr is very uncommon.

Aims: We present here a case of FVL in the absence of APCr in a patient who underwent liver transplantation.

Methods: Forty-six-year-old female underwent successful orthotopic liver transplantation for HBV-related liver failure and was referred to our Centre for thrombophilia testing due to positive family history of venous thromboembolism (VTE). Laboratory tests for antithrombin activity, protein C activity and free protein S antigen level were performed. APCr assay (with predilution with FV deficient plasma) was determined by using Coatest® APC Resistance V Kit (Chromogenix). Molecular screening for common prothrombotic mutations [c.1601G>A in F5 and c.*97G>A in prothrombin gene] was carried out using PCR-RFLP. F5 gene analysis was also performed by direct sequencing of exon 10 on the ABI 3130XL Genetic Analyzer (Applied Biosystems).

Results: The patient have not had a personal history of VTE. Laboratory tests revealed heterozygous FVL despite of normal APC response in plasma. Other thrombophilic defects were ruled out. Testing of family members confirmed presence of heterozygous FVL in one asymptomatic patient's son. We believe that in this case the normal APC response in APCr test results from production of normal coagulation factor V by donor liver, whereas FVL represents the patient's genomic DNA isolated from leukocytes.

Conclusion: This case demonstrates that interpretation of thrombophilia testing in a patient after liver transplantation may be more complex than in other patients groups. The clinical significance of our findings has yet to be explained.

Disclosure of Interest: None declared.

PO544-TUE

Venous thromboembolism associated with first pregnancy in 80 women with hereditary antithrombin deficiency

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Background: The risk of venous thromboembolism (VTE) associated with antithrombin deficiency (AT-Def) during pregnancy is not well defined and recent recommendations for antithrombotic prevention have been discussed.

Aims: We report a retrospective study of VTE during the first pregnancy of 80 women (W) with AT-def (67 families).

Methods: The type of AT-Def was classified according to AT activity, antigen concentration, and *SERPINC1* mutation: A type I quantitative is present in 56 W, type II qualitative in 24 (reactive site in 1, pleiotropic in 6, heparin binding site (HBS) in 16 and Cambridge in 1 W). A concomitant thrombophilia was present in 10 W (2 prothrombin G20210A and 8 Factor V Leiden mutations). The 16 W with type II HBS were analysed separately, since this mutation is associated with a lower risk of VTE.

Results: Among the 64 W with AT-def non HBS, aged $28.2a \pm 4.7$ (17–39) years, 43 W were asymptomatic before the pregnancy and 21 had a previous VTE. A first VTE was observed in 13 of the 27 W (48%) without prevention, and in 1 of the 13 treated W (18%). A recurrent VTE occurred in 5 W with a previous VTE (4 of the 18 receiving a therapeutic anticoagulant treatment during pregnancy and 1 of the 3W without treatment). In total, among the W with AT-def non HBS, a pregnancy related VTE is observed in 16% (5/31) of the treated W and 47% (14/30) of the W without prevention ($P = 0.02$). In the group of 16W with type II HBS, 14 were asymptomatic before pregnancy, 4 received a prevention and only 1 VTE was observed. A

multivariate analysis show a significant association of VTE associated pregnancy with the type of the deficiency (non HBS vs. HBS, $P = 0.007$), the absence of prevention ($P = 0.001$) and the presence of a concomitant inherited thrombophilia ($P = 0.01$).

Conclusion: Our results confirms the high risk of a first-pregnancy related VTE in AT deficiency non type IIHBS in the absence of prevention, and are in favour of a therapeutic or preventive LMWH during pregnancy in these patients with or without previous VTE.

Disclosure of Interest: None declared.

PO545-TUE

Molecular modeling of a novel mutation in the *SERPINC1* gene associated with type 1 antithrombin deficiency

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Background: Antithrombin (AT) deficiency is a rare thrombophilia. Molecular modeling can help characterize new variants.

Aims: To describe a novel mutation in the *SERPINC1* gene resulting in type 1 AT deficiency, with structural visualization and in-silico stability simulations performed to confirm pathogenicity.

Methods: The *SERPINC1* gene was analyzed by PCR amplification followed by sequencing of both DNA strands. The solvent accessible surface area for AT was estimated using utilities in PyMOL, a 3D molecular visualization program, with the default parameters. The electrostatic surface area was calculated with the Adaptive Poisson-Boltzmann Solver. Rosetta, a program developed by the Baker laboratory predicts lowest energy protein structures. The $\Delta\Delta G$ application of Rosetta semi-quantitatively estimates the change in protein free energy due to a single mutation. The resultant energy term is of arbitrary units (reu), but a positive value indicates that the protein has been destabilized by the mutation.

Results: A 27-year-old female presented with left-leg deep vein thrombosis. Thrombophilia workup revealed a low AT activity (38%). Three months after stopping anticoagulation, AT activity remained low (41%), with an antigen level of 46%. Genetic sequencing identified the heterozygous variant, c.1196C>A in exon 6 of the *SERPINC1* gene, resulting in the amino acid substitution A399E. The AT X-ray structure used for simulation, 1T1F, was idealized by Rosetta to remove any potential crystal artifacts before the energy calculation. The $\Delta\Delta G$ for A399E is predicted to be +9.0 reu, indicating that the protein is destabilized by the mutation. A399E buries a negative charge in the core of AT with zero accessible surface area. Based on the burial of the charge and the $\Delta\Delta G$ simulations of charged residues, AT is most likely destabilized by the A399E mutation.

Conclusion: The novel c.1196C>A variant in the *SERPINC1* gene is pathogenic for AT deficiency. Molecular modeling is useful in determining the pathogenicity of new variants.

Disclosure of Interest: None declared.

PO546-TUE

Antiphospholipid antibodies as embolic risk marker of infective endocarditisDevignes J¹, Maigrat C-H², Goehringer F³, Thivillier C⁴, Juillière Y², Lecompte T⁵, Doco-Lecompte T⁶ and Selton-Suty C⁷

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Background: Infective endocarditis (IE) is a rare but serious disease with a mortality rate of about 20%. Embolic complications, including those of brain, are associated with significant morbidity and mortality. **Aims:** The aim of our study was to reassess the relationships between antiphospholipid antibodies (aPL) and the embolic events occurring in the IE.

Methods: Between 2007 and 2012, 175 adults with IE (Duke-Li classification) were prospectively included. Among them, 110 (63%) underwent an embolic event, including 47 brain embolisms. In 74 cases the embolic event was diagnosed after IE diagnosis. The presence of aPL was assessed by ELISA tests with cardiolipin (aCL IgG/IgM, European forum recommendations, Tincani 2001) and β 2-glycoprotein I (anti- β 2GPI IgG/IgM, Alegria[®], Orgentec). The occurrence of embolic events after IE diagnosis with or without aPL was assessed by the Kaplan-Meier method and the log-rank test (SAS software 9.3).

Results: Among the 175 IE patients, 31 (18%) had at least one positive finding for aPL (19 with isotype IgM). Overall, patients with anti- β 2GPI IgM were more frequently subject to brain embolism than those without ($P = 0.033$); in multivariate analysis, associated factors were aCL IgM (OR = 2.68; 95% CI 1.15 to 6.24; $P = 0.02$) and anti- β 2GPI IgM (OR = 4.55; 95% CI 1.71 to 12.1; $P = 0.002$). According to Kaplan-Meier curves, the occurrence of embolic events after IE diagnosis was associated with anti- β 2GPI IgM ($P = 0.0035$), and the occurrence of brain embolism was associated with aCL and anti- β 2GPI IgMs ($P < 0.0001$ and $P = 0.0037$ respectively).

Conclusion: This study suggests a link between the anti-aCL or - β 2GPI IgMs and embolic events in IE. The analysis of these markers could help identifying patients at high risk for embolic complications and considering emergency surgery. Otherwise, the detection of anti- β 2GPI in this context and the involvement of isotype IgM but not IgG is questioning some dogmas about aPL.

Disclosure of Interest: None declared.

PO547-TUE

Hereditary and acquired thrombophilias and womenElezovic I¹, Antic DA² and Mitrovic M³

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Background: Women with hereditary thrombophilia (HT) and/or acquired thrombophilia due to antiphospholipid antibodies (APA) are at an increased risk of venous thromboembolism (VTE), miscarriages and obstetric complications.

Aims: Acquired and HT were tested at 987 women, aged 9–76 years (Med = 32), with thrombosis, miscarriages, obstetric complications, or with positive family history.

Methods: Deficiency of antithrombin, protein C, protein S, factor XII, and lupus anticoagulant measured with coagulation tests, ACA and anti β 2GPI antibodies with ELISA and FV Leiden, prothrombin 20,210, MTHFR and PAI-1 4G variant with PCR methods.

Results: Results showed HT at 873 women and 226 with APA. One HT detected in 426/761 women (56%), and two or more in 335/761 (44%). In 112/873 women (13%) with HT simultaneously were detected APA. At 112/226 women with APA HT were detected (50%). Thrombosis has had 176/761 (23%) of women with HT, 44/112 (39%) with HT and APA, and 49/114 (43%) with APA alone. Two or more thrombosis had 66/176 (37%) of women with HT and 26/49 (53%) with APA. This difference is significant. Two or more miscarriages have had 509/761 (67%) women with HT, 48/114 (42%) with APA and 65/112 (58%) with both. During pregnancy 217 women with previous 313 miscarriages, after diagnosed of thrombophilias were treated prophylactically with LMWH $\pm$ folic acid (for MTHFR). They delivered 244 babies, and 24 of them during pregnancy were diagnosed and treated for thrombosis. In addition, 28 women have had 32 successfully delivery after 79 unsuccessful *in vitro* fertilisations.

Conclusion: Intensively examines the efficacy of LMWH in the prevention of recurrent miscarriage in women with HT and/or APA, is confirmed by our results, as well as efficiency of prevention and treatment of VTE in pregnancy.

Disclosure of Interest: None declared.

PO548-TUE

Characterization of a new hereditary prothrombin variant associated with increased risk of venous thromboembolism

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Background: Proband from two unrelated Italian families and with a family history of thromboembolism developed recurrent deep vein thrombosis at young age. We did not identify any known cause of hereditary thrombophilia and coagulation tests were normal except for a decreased prothrombin clotting activity. We identified a new prothrombin mutation (c.1786C>T, p.R596W), named prothrombin Padua 2.

Aims: To understand how this novel mutation may alter the functional properties of thrombin and cause thrombosis.

Methods: To assess the effect of the mutation on thrombin inactivation by antithrombin (AT), plasma of probands were incubated with phospholipids, calcium and ecarin to allow the conversion of prothrombin to thrombin and then treated with excess AT for different times. A chromogenic thrombin substrate was added and changes in absorbance per min were measured at 405 nm. Relative residual thrombin activity (RRTA) was calculated by comparison with 0-min data. To explore the effect of the mutation on thrombin generation, a thrombin-generation assay was performed using calibrated automated thrombography before and after the addition of excess AT to plasma samples. Plasma from a healthy family member was used as control.

Results: After 30 min of inactivation by AT, the RRTA in the plasma of probands were significantly higher (61.1% and 58.0%, respectively) than those in 2 healthy relatives (28.4% and 35.1%, respectively). The thrombin-generation assay in the presence of AT150% showed a reduced maximum thrombin concentration (peak) and a prolonged start tail in the plasma of probands in comparison to the controls. Nodifference was detected in the endogenous thrombin potential.

Conclusion: These data, obtained in plasma, suggest that prothrombin Padua 2 mutation may cause antithrombin resistance and therefore predispose to thrombosis, as well as prothrombin Yukuhashi (p.R596L) and prothrombin Belgrade (p.R596Q). Arginine at position

596 of the prothrombin molecule might be a hot spot for mutations in thrombophilic patients.

Disclosure of Interest: None declared.

PO549-TUE

Arteriovenous thrombosis in end stage renal disease patients

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Background: Renal disease can result in significant disorders of haemostasis. Alterations in haemostatic plasma protein factors have been suggested to create a hypercoagulable state in End Stage Renal Disease (ESRD) patients and vascular access thrombosis (VAT) represents a serious and common problem in these patients

Aims: The aim of this study was to determine the frequency of thrombotic complications and to identify factors associated with arteriovenous thrombosis in ESRD patients receiving renal replacement therapy with age matched control group of ESRD who never had a thrombotic occlusion

Methods: One hundred and twenty ESRD patients on regular dialysis, having recent renal transplant, were selected for the study. This sample was divided into two groups; a case group of 61 patients who had arteriovenous thrombosis with temporary central lines thrombosis and vascular access problems and a control group of 59 ESRD patients, who never had a VAT. Known cases of congenital or acquired thrombotic disorders were excluded. Findings were recorded in a structured questionnaire. Laboratory analysis was done after clinical and radiological evaluation. Thrombophilia screening included Antithrombin (AT), protein C (PC), protein S (PS) deficiencies and lupus anticoagulant (LA) in both groups.

Results: In case group, forty-seven out of 61 patients selected were positive for thrombophilia screening with PC deficiency in 26.2%, PS deficiency in 16.3%, AT in 5%, LA in 23.1% and combined deficiency was observed in 16.3%. In control group, PC was 15.1%, PS 11.1% and AT 1.5% results show no significant influence from deficiencies of the endogenous anticoagulant proteins on VAT. There was no statistically significant difference between cases and controls regarding these factors except LA 20.2%. LA demonstrated a significant difference on VAT ($P = <0.05$)

Conclusion: Our study demonstrated a significant lupus anticoagulant association with increased risk of VAT. Large cohort studies are needed to confirm this finding.

Disclosure of Interest: None declared.

Tissue factor and factor VII – II

PO550-TUE

Effect of a carboxy-terminal peptide on factor VIIa activity

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Background: OPKO is developing a long acting version of therapeutic proteins utilizing CTP technology. This involves fusion of the hCG C Terminus Peptide to proteins. The technology was clinically validated for several drugs while maintaining their biological activity.

Aims: The aim of this study was to assess the effect of the CTP on factor VIIa activity.

Methods: rFVII-CTP was expressed in CHO cells, purified utilizing a CTP specific purification process, and activated. Activity was measured as the ability to activate factor X; these assays were done with and without tissue factor (TF). TF binding was assessed by varying factor VIIa. Kinetics of factor X activation were assessed by varying factor X. Platelet surface binding and activity was assessed by incubating with FVIIa or FVIIa-CTP, then adding plasma levels of FX and prothrombin and measuring thrombin generation. Antithrombin (AT) inhibition of the TF complex was measured in the presence of heparin. TFPI inhibition was assessed by competition with FX.

Results: rFVIIa-CTP was comparable to rFVIIa in binding to TF. Once bound to TF, rFVIIa-CTP was near normal (> 90%) in ability to activate factor X; the small decrease was due to a difference in Vmax and not a change in Km for factor X. rFVIIa-CTP had normal ability to bind to phospholipid vesicles. Once bound rFVIIa-CTP activated factor X at about half the rate of rFVIIa. This effect was a change in Vmax, the Km toward factor X was normal. Thrombin generation on platelets was slightly slower in the presence of rFVIIa-CTP than rFVIIa. In the presence of TF, AT, and heparin, rFVIIa-CTP retained activity slightly longer than FVIIa with a change in the rate of inhibition of just over half. Inhibition of the TF complex by TFPI was normal for rFVIIa-CTP.

Conclusion: rFVIIa-CTP has normal activity with TF but reduced TF-independent ability to activate factor X relative to rFVIIa. The biochemical assays are consistent with the observation that rFVIIa-CTP is effective at stopping bleeding in animal models.

Disclosure of Interest: D. Monroe Grant/Research Support from: OPKO Biologics, L. Binder Employee of: OPKO Biologics, M. Hoffman Employee of: OPKO Biologics, G. Hart Employee of: OPKO Biologics, M. Hoffman Grant/Research Support from: OPKO Biologics.

PO551-TUE

A 28-years-old woman with factor VII deficiency revealed after two severe post operational bleeding episodes

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Background: Congenital factor VII (FVII) deficiency is a rare autosomal recessive hemorrhagic disorder. Its clinical spectrum varies from asymptomatic to lethal hemorrhage.

Aims: We report a new case of congenital FVII deficiency from Greece.

Methods: Our case is a 28 y/o woman. She reports two vaginal deliveries with a severe bleeding in one. Recently, she underwent surgical removal of a benign breast tumor. Eight and 11 days post-operation she experienced two serious bleedings from the surgical wound that were treated conservatively and surgically, respectively. An oozing type of bleeding was observed. No hemorrhagic family history was reported. Patient's clotting profile included prothrombin time PT, activated partial thromboplastin time aPTT, plasma fibrinogen levels and determination of coagulation factors activity all by the use of commercial kits (SIEMENS Healthcare diagnostics). The method used for the molecular analysis was the direct sequencing, utilizing the Sanger technique.

Results: Patient's clotting profile showed prolonged PT of 16.9 s (range 9.5–12), normal aPTT (28.3 s), fibrinogen levels and platelets counts. FVII-deficiency with 42% activity (range 70–120%) and a normal activity for FII, FV and FX were confirmed. An inhibitor screen-

ing by mixing with normal human plasma showed correctable PT. Molecular analysis revealed that our patient was heterozygous for both p.Ala354Val and p.Pro464HisfsX28 mutations.

Conclusion: The potential link between mild FVII-deficiency and the bleeding phenotype is unclear albeit classical bleeding causes such as platelet defects and Willebrand disease were excluded. Both mutations have been previously described on the same allele in FVII-deficient patients from Eastern and Northern Europe, but not in our region. Considering the lack of epidemiological data, we think that the recording of a new case will enrich the molecular epidemiology of congenital FVII deficiency in Greece.

Disclosure of Interest: None declared.

PO552-TUE

Tissue factor/FVIIa transactivates the IGF-1R in prostate cancer cells via inhibition of caveolin-1

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Background: We recently described a tissue factor (TF)/factor VIIa-dependent transactivation and nuclear accumulation of the receptor tyrosine kinase IGF-1R. Caveolae are well characterized cell membrane signaling compartments, but their role in TF signaling is poorly understood.

Aims: To clarify the mechanism behind the TF-induced phosphorylation of the IGF-1R, we investigated the interaction between IGF-1R and caveolin-1 (cav1, the principal protein in caveolae), in FVIIa-treated prostate cancer cells.

Methods: Prior incubation with FVIIa, PC3 cells were treated with: cav1 siRNA, scramble siRNA, a peptide corresponding to cav1 aminoacids 82–101 (the scaffolding domain), or with a scramble peptide. The phosphorylations of IGF-1R and cav1 were determined using Duolink In Situ and western blot (WB), and the nuclear localization of the IGF-1R was assessed by WB on fractionated cell lysates.

Results: FVIIa treatment (10 and 100 nM) increased the phosphorylation of the IGF-1R after 30 min and induced a nuclear translocation after 2 h. The IGF-1R was also activated by cav1 siRNA knockdown. This indicated an inhibitory role of cav1 on the IGF-1R in resting cells and the addition of FVIIa was not able to further phosphorylate the IGF-1R in the cells with reduced expression of cav1. Additional experiments showed that pretreatment with the cav1 scaffolding domain peptide completely abolished the effects of FVIIa (100 nM) regarding IGF-1R phosphorylation and nuclear translocation. The formation of the TF/FVIIa-complex did not alter cav1 protein levels but induced a phosphorylation of tyrosine 14 on cav1 after 10 min.

Conclusion: In summary, we show that cav1 inhibits the activation of the IGF-1R via its scaffolding domain, and that it becomes phosphorylated on tyrosine 14 after treatment with FVIIa. We suggest that the cav1 phosphorylation terminates the inhibition of cav1 on the IGF-1R, and that it thereby serves as a crucial step in the TF/FVIIa-dependent IGF-1R transactivation.

Disclosure of Interest: None declared.

PO553-TUE

The LRP1 receptor promotes the *in vivo* clearance of activated factor VII (FVIIa) in complex with antithrombin

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Background: Activated factor VII (FVIIa) is approved for the treatment of several bleeding disorders, including hemophilia with inhibitors. After intravenous injection, its half-life (T_{1/2}) is relatively short (2–3 h), which limits its application in prophylactic therapy. FVIIa is cleared predominantly (~65% of the injected protein) following complex formation with the Serpin antithrombin (AT; Agersø JTH 2011-9:333). FVIIa/AT complexes are targeted to hepatocytes and liver macrophages, *i.e.* Kupffer cells (Seested Thromb Res 2011-127:356). Both cells lines abundantly express LRP1, a scavenger receptor involved in the clearance of protease/Serpin complexes.

Aims: To investigate whether (i) FVIIa/AT is a ligand for LRP1 and (ii) LRP1 modulates clearance of administered FVIIa.

Methods: FVIIa clearance was determined in macrophage LRP1-deficient mice (macLRP1-). Binding of FVIIa and FVIIa/AT to macrophages and purified LRP1 was performed.

Results: Survival of FVIIa was prolonged 2-fold in macLRP1-deficient mice compared to control mice [0.9 h (95% CI 0.7–1.5 h) vs. 0.4 h (95% CI 0.3–0.6 h); $P < 0.001$], and recovery was modestly increased ($44 \pm 5\%$ vs. $36 \pm 4\%$; $P < 0.05$). Cell-binding experiments revealed binding of both FVIIa and FVIIa/AT to THP1-macrophages. FVIIa binding was reduced in the presence of annexin-V or an anti-tissue factor antibody, while insensitive to the LRP1-antagonist RAP. In contrast, binding of FVIIa/AT was selectively inhibited by RAP. Indeed, binding assays using purified proteins demonstrated that FVIIa/AT, but not FVIIa alone, bound in a specific dose-dependent and saturating manner to LRP1 or a recombinant ligand-binding LRP1 fragment (Cluster IV).

Conclusion: Our data indicate that the FVIIa/AT complex but not FVIIa alone is a ligand for LRP1, and LRP1 also may contribute to the clearance of the FVIIa/AT complex.

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PO554-TUE

High plasma concentration of activated factor vii-antithrombin complex is associated with an enhanced thrombin generation

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Background: Plasma concentration of activated factor VII (FVIIa)-antithrombin (AT) complex has been proposed to reflect tissue factor (TF) exposure, thus being a potential biomarker of a prothrombotic diathesis. However, the clinical and laboratory significance of FVIIa-AT remains still elusive.

Aims: To investigate the possible correlations between FVIIa-AT plasma concentration and thrombin generation parameters, *i.e.* Lag Time, Time to Peak, Peak, and Endogenous Thrombin Potential (ETP).

Methods: The analyses were performed in 272 subjects (206 males and 66 females, mean age 59.8 ± 11.1 years, 198 with and 74 without coronary artery disease (CAD). Subjects who were taking anticoagulant drugs, who had acute coronary syndrome or any acute illness in the month preceding the enrolment, or who had severe renal and/or liver impairment, were excluded from this study. The plasma concentration of FVIIa-AT was measured by ELISA. Thrombin generation was assessed by means of a fluorogenic method.

Results: FVIIa-AT plasma concentration correlated directly with Peak ($R = 0.143$; $P = 0.018$) and ETP levels ($R = 0.143$; $P = 0.018$), while no association was found for Lag Time ($R = 0.066$; $P = 0.276$) and Time to Peak ($R = -0.052$; $P = 0.395$). Subjects with FVIIa-AT levels higher than the median value (> 79 pM) had increased Peak (6.82 (6.55 – 7.03) vs. 6.36 (6.17 – 6.55) Rfu/s; $P = 0.009$) and ETP levels (906.9 (880.1 – 925.2) vs. 854.1 (837.2 – 871.3) Rfu; $P = 0.001$) in comparison with those having lower levels. A high FVIIa-AT plasma concentration remained a significant predictor of Peak (b-coefficient= 0.168 ; $P = 0.006$) and ETP levels (b-coefficient= 0.200 ; $P < 0.001$) after adjustment for sex, age, and CAD diagnosis, and even including the traditional coagulation times in the regression model.

Conclusion: In this study high FVIIa-AT levels were consistently associated with thrombin generation, in particular with an increased ETP, thus supporting the hypothesis of FVIIa-AT as a marker of hypercoagulability.

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PO555-TUE

Tissue factor and factor VII gene polymorphisms influencing FVIIa-AT plasma concentration are not uniformly associated with mortality in patients with stable coronary artery disease

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Background: Plasma concentration of activated factor VII (FVIIa)-antithrombin (AT) complex has been proposed to reflect tissue factor (TF) exposure, thus being a biomarker of a prothrombotic diathesis. We have previously demonstrated that high FVIIa-AT levels may be an independent predictor of total and cardiovascular mortality in patients with stable coronary artery disease (CAD).

Aims: To investigate TF and F7 gene polymorphisms as determinants of FVIIa-AT plasma concentration and their potential association with mortality in the setting of secondary prevention of CAD.

Methods: Within the original selection of Verona Heart Study subjects for whom FVIIa-AT data were available ($n = 686$, 546 with and 140 without angiographically-proven CAD) and according the availability of DNA samples, genotyping of TF (-603 A>G and $+5466$ A>G) and F7 (-402 G>A, -323 A1/A2 and R353Q) polymorphisms was performed.

Results: TF -603 A>G polymorphism was significantly associated with FVIIa-AT plasma concentrations, with GG-homozygotes having lower levels than carriers of A allele. F7 -323 A1/A2 and R353Q genotypes correlated significantly with FVIIa-AT levels according with the influence on FVIIa. In a multivariate adjusted model TF -603

A>G and F7 -323 A1/A2 polymorphisms remained significant predictors of FVIIa-AT variability. By Kaplan-Meier survival analysis only TF -603 A>G polymorphism was a predictor of mortality in the CAD population, while no significant association was found for the other genetic variants. TF -603 GG-homozygotes were associated with a decreased risk of both total (HR 0.52 with 95% CI 0.30–0.93) and cardiovascular mortality (HR 0.45 with 95% CI 0.21–0.95) in sex- and age-adjusted Cox-regression models.

Conclusion: The different association of FVIIa-AT genetic determinants with mortality suggests that the mechanisms causing the increase in plasma concentration of FVIIa-AT (and not only the levels) may have a key role in CAD prognosis, thus reviving the interest in the TF pathway.

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PO556-TUE

The role of factor VII mutations in endoplasmic reticulum stress

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Background: Inherited coagulation factor VII (FVII) deficiency is caused by heterogeneous mutations in the F7 gene and is associated with increased bleeding risk, inversely correlated with plasma FVII levels. The FVII mutations L204P, Q100R, G360V, del229I and A294V-11125delC, most of which are associated with life-threatening bleeding symptoms, impair FVII intracellular trafficking and secretion, possibly due to misfolding. Accumulation of misfolded proteins within the endoplasmic reticulum (ER) cause ER stress and can trigger the unfolded protein response (UPR) and apoptosis.

Aims: To investigate ER stress responses in cells expressing the various mutant FVII proteins.

Methods: Chinese hamster ovary (CHO-K1) cells were transiently transfected with plasmids expressing the wild-type (wt) or mutant FVII. ER stress was measured using the Cignal ERSE reporter Luciferase assay. Upregulation of ER chaperones and transcription factors and activation of kinases was measured using Western blotting. The apoptotic effect of FVII mutants was measured in CHO-K1 cells using the Caspase-Glo[®] 3/7 luminescent assay and flow cytometry.

Results: The relative Luciferase activity was higher in cells expressing the mutant FVII compared to wt, suggesting an increased ER stress response. Moreover, we observed a small increase in caspase 3/7 activity in the FVII mutant-expressing cells compared to the wt indicating increased apoptotic activity. ER stress studies using Western blotting and studies on apoptosis using flow cytometry, are currently in progress.

Conclusion: These findings suggest that FVII mutations, which change, delete or extend the FVII sequence, induce ER stress most likely due to accumulation of misfolded proteins. Alternative therapies targeting the underlying pathogenic molecular mechanism of the FVII mutations would be promising for the patients compared to the current replacement therapy.

Disclosure of Interest: None declared.

PO557-TUE

FVII deficiency: unveiling the cellular and molecular mechanisms underlying three model alterations (missense, deletion, extension) of the FVII catalytic domain

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Background: The inherited coagulation factor VII (FVII) deficiency is the most common amongst rare bleeding disorders. Although several mutations have been characterized, the intracellular mechanisms responsible for severe type I deficiency forms, the most frequent, are still unclear. At present, treatment of FVII deficiency is based on replacement therapies, which has limitations.

Aims: To reveal cellular and molecular pathogenic mechanisms of three model molecular defects of the FVII catalytic domain: the G360V missense change, the one-residue deletion del229I and the frame-shifted and elongated variant A294V-11125delC, all associated with severe bleeding.

Methods: The wild type (wt) and mutant FVII were expressed in CHO-K1 cells, the protein levels were measured in medium and cell lysates (CL) by ELISA and Western blot (WB) and FVII activity by fluorogenic assays. Stability of FVII variants was evaluated by pulse-chase stable isotope labelling followed by mass spectrometry based quantitative proteomics. Intracellular localization was assessed by confocal microscopy. Molecular modelling was performed to describe the possible atomic consequences of the mutations.

Results: FVIIwt and mutants were detected in CL, but only FVIIwt was appreciably secreted. Intracellular studies revealed that the FVII mutants accumulated in endoplasmic reticulum without reaching the Golgi apparatus. The FVII synthesis and stability patterns seemed to differ between the wt and mutants and also amongst the mutants. Molecular modeling suggested that the G360V substitution might cause severe structural disruption of the molecule.

Conclusion: These findings suggest an impaired intracellular trafficking of FVII mutants, possibly due to misfolding, which seems to play a role in the stability of the mutants. Therapies targeting the molecular mechanism of the mutants would be promising for the patients as even a modest increase of functional protein will be beneficial.

Disclosure of Interest: None declared.

PO558-TUE

microRNAs modulate human tissue factor pathway inhibitor-A (TFPIA) expression in response to androgens in endothelial cells

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Background: TFPI expression in adults is regulated by 5 α -dihydrotestosterone (DHT). This regulation contributes to the reduction of this anticoagulant from the 50's and might play a role in the increased risk for cardiovascular events. The molecular mechanisms underlying are unknown.

Aims: To evaluate the role of miRNAs on the regulation of TFPIa expression in response to DHT.

Methods: Endothelial cells (EA.hy926 and HUVEC) were transfected with precursors and inhibitors of miRNAs. TFPIa expression was quantified by qRT-PCR and western blot, and miRNA levels by qRT-PCR. The validation of these results was performed by analyzing miRNA and TFPI expression in 74 HUVEC samples from human umbilical cords. EA.hy926 cells were treated with physiological doses of DHT (30 nM, 24 h and 72 h). TFPI anticoagulant activity in EA.hy926 and HUVEC supernatants was determined with Actichrome[®] TFPI activity assay kit (Sekisui).

Results: *In silico* studies allow the selection of 4 miRNAs as potential TFPIa regulators. Only miR-27a/b precursors significantly decreased TFPI mRNA (NM_006287) and protein and, miRNA inhibitors yielded the opposite result in two endothelial cell lines. Luciferase assays demonstrated a direct interaction between miR-27a/b and TFPI 3'UTR. *Ex vivo* study showed a significant and inverse correlation between TFPI mRNA and miR-27a levels. Anticoagulant activity of TFPIa decreased ~30% with miR-27a and increased ~50% with anti-miR27a. Interestingly, DHT treatment induced a significantly increased (~40%) TFPIa expression (of both mRNA and protein) with a parallel decreased (~50%) of miR-27a/b expression.

Conclusion: miR-27a/b directly regulate TFPIa expression in endothelial cells, which influences TFPI anticoagulant activity. In addition, our results suggest that DHT is a hinge in miR-27/TFPIa regulation axis. Future studies are needed to investigate whether DHT variations are involved on a miR27/TFPIa dysregulation that could increase the cardiovascular risk inherent to aging. Disclosure of Interest: None declared.

PO559-TUE

Single centre experience of factor VII deficiency

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Background: We report on 62 patients who have been referred to our Centre for evaluation of FVII deficiency over the last 20 years. Of these 31 patients underwent investigations because of a history of bleeding, 11 were tested as part of family study and 19 were investigated because of an incidental finding of prolonged INR on preoperative coagulation screening.

Aims: To establish whether there is any correlation between FVII levels, genotype and bleeding tendency and to review the surgical management of patients in a large cohort of patients from a single centre.

Methods: A retrospective review was conducted of patients' records with a view to eliciting bleeding history, F VII levels, *F7* genotype and haemostatic cover.

Results: The 62 patients ranged in age from 5 to 88 years: 26 were male and 36 were female. Only two patients were classified as having severe FVII deficiency with a baseline FVII level <1 iu dL⁻¹. The remainder had baseline FVII level ranging from 1 to 49.2 iu dL⁻¹. 31/62 patients gave a bleeding history. One of the two patients with severe F VII deficiency presented with epistaxis and the other with menorrhagia and epistaxis. No instances of severe bleeding were observed in the entire cohort. The *F7* gene was analysed in 52/61 patients, and of these 51 had either a pathogenic mutation or polymorphisms which would explain their FVII level. There was poor correlation between genetic variant and levels.

Conclusion: Eleven patients with FVII level ranging from <1 iu dL⁻¹ to 25 iu dL⁻¹ received rVIIa concentrate to cover one or more major surgical procedures. Minor surgical procedures were successfully managed with tranexamic acid alone, with rVIIa on hand in case of bleeding. Results show that there was poor correlation between phenotypic FVII levels, *F7* genotype and bleeding tendency. Replacement with rVIIa concentrate is required only in selected cases and is effective at securing haemostasis

Disclosure of Interest: None declared.

PO560-TUE

Thrombin generation in patients with mild factor VII deficiency measured in the presence of thromboplastins of different origin

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Background: Factor VII (FVII) deficiency is a rare congenital bleeding disorder with mostly mild symptoms.

Aims: Thrombin generation (TG) was measured in a cohort of patients with mild FVII deficiency in the presence of thromboplastins (TP) of different origin. The aim was to compare TG in patients with FVII deficiency and in controls and associate the bleeding symptoms and levels of FVII with the haemostatic potential of the patients.

Methods: TG was measured by the Calibrated Automated Thrombogram (CAT®) in platelet poor plasma in the presence of 5 pM tissue factor (human recombinant, h-TP) and in the presence of a corresponding rabbit brain TP (r-TP) concentration. 4 µM phospholipids were used in both measurements. The TG markers measured were lagtime, endogenous thrombin potential (ETP), peak and time to peak (tpeak). Thrombin generation was measured in nine patients with mild FVII deficiency and in twelve healthy controls. Only one patient had a severe bleeding phenotype, while the others had very mild or no symptoms.

Results: The median FVII activity was 0.55 kIU L⁻¹ (0.32–1.03) (in the presence of h-TP) and 0.29 kIU L⁻¹ (0.19–0.54) (in the presence of r-TP). In the presence of h-TP, ETP and tpeak were higher in the control group (ETP: $P = 0.028$, 1971 nM*min and 1700 nM*min resp., tpeak: $P < 0.001$). In the presence of r-TP, lagtime, peak and tpeak were higher in the control group (lagtime: $P = 0.001$, peak: $P = 0.004$, 328 nM and 199 nM resp., tpeak: $P = 0.003$). TG did not differ significantly among the patients with FVII deficiency. There was no clear association between TG markers, FVII levels and bleeding phenotypes.

Conclusion: ETP in the patient group was not markedly decreased compared to controls, even for the patient with severe bleeding symptoms. In conclusion, regardless of FVII levels, TG was lower, but within the reference range (as defined for this laboratory) in this cohort of patients with mild FVII deficiency and mostly mild bleeding symptoms, as compared to controls.

Disclosure of Interest: None declared.

PO561-TUE

Mutations in the *F7* gene in Swedish patients with discrepant factor VII activity in the presence of thromboplastins of different origin

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Background: Factor VII (FVII) deficiency is the most common among the rare congenital bleeding disorders. Some mutations in the *F7* gene cause differences in the levels of FVII when thromboplastins of different origin are used.

Aims: This study was initiated when it was observed that in ten patients with mild FVII deficiency referred to the coagulation laboratory of the university hospital in Linköping, the ratio [FVII activity measured in the presence of human/rabbit thromboplastin, TP] was > 1.25 (i.e. the median ratio for this laboratory). The aim of the study was to investigate whether a novel mutation in the *F7* gene could be responsible for this finding.

Methods: The *F7* gene for every patient was analysed by polymerase chain reaction (PCR) and bidirectional sequencing of the coding region and exon-intron splice junctions. In addition, a multiplex ligation-dependent probe amplification assay was performed to test for the deletion or duplication of one or more entire exons.

Results: The cohort consisted of seven female and three male patients. The median age was 45.5 (20–86) years. The median FVII activity was 0.55 kIU L⁻¹ (0.32–1.03) (in the presence of human recombinant TP) and 0.29 kIU L⁻¹ (0.19–0.54) (in the presence of rabbit brain TP). Nine out of ten patients had one mutation in common (Arg³⁵³Gln). The ratios were highest in three patients; two of those were heterozygous for FVII Shinjo and one was heterozygous for FVII Padua.

Conclusion: The Arg³⁵³Gln polymorphism which was found in 90% of the patients in this cohort is rather common, as previously reported in blood donors. The prevalence in the Swedish population is unknown. The most prominent differences in ratios between human and rabbit thromboplastin were observed in the patients with FVII Padua and FVII Shinjo, which are known to produce such variations due to source of thromboplastin. It was thus concluded that the Arg³⁵³Gln mutation did not contribute significantly to the observed discrepancies.

Disclosure of Interest: None declared.

PO562-TUE

FVII deficiency and thrombosis, coincidence or new mechanism of thrombophilia

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Background: A large number of arterial and venous thromboembolic events were reported in hemorrhagic coagulopathies such as in hemophilia A and B, von Willebrand disease and factor VII deficiency.

Aims: We propose in this presentation a review of literature and a case report of a FVII deficiency associated with recurrent thrombosis.

Methods: Phenotypic tests of coagulation were performed on STA compact- Stago. DNA extraction by Quick-Gene mini-80 - Fujifilm. The FVII mutation researched by direct sequencing.

Results: We report a 34-year-old male patient with the antecedent of recurrent phlebitis and cerebral venous thrombosis. The investigations revealed only a deranged prothrombin time (18"/12") without any abnormalities of the thrombophilia testing, no inhibitors deficiency, no lupus anticoagulants, no increase level of FVIII c, FV and FII polymorphisms are negatives. Prothrombin complex testing revealed an isolated FVII deficiency at 26%. The direct sequencing of F 7 revealed a homozygous mutation 'C.-56C>T' never reported in the databases and located near the transcription factor binding site HNF4. According to Mariani, two hypothesis can be formed, the first consists on the direct effect of FVII's mutation in the hypercoagulation with a non-response of F VII-TF -F X complex to the inhibition by TFPI, the second is the association of F VII deficiency to other risk factors that must be searched. On the European journal of Hematology 2012 Girolami analyzed all published cases and precised that the association of thrombosis risk factors was found only in 13 cases out of 33. In our case, the *in Silico* analysis have confirmed the asymptomatic character of our patient (absence of hemorrhage) and the implication in the thrombotic events remains to be elucidated.

Conclusion: The existence of even isolated cases of thrombosis associated with FVII deficiency, suggests that this deficit does not protect against the risk of thromboembolism, involvement is still to be confirmed.

Disclosure of Interest: None declared.

TTP / thrombotic microangiopathies II

PO563-TUE

Use of a plasma derived FVIII product for primary prophylactic therapy in a infant with congenital thrombotic thrombocytopenic purpura

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Background: Congenital thrombotic thrombocytopenic purpura (cTTP) is rare and difficult to diagnose. Optimal therapy is not yet available as patients need plasma infusion therapy to avoid end-organ microthrombotic complications.

Aims: We describe a child with cTTP, first diagnosed with neonatal alloimmune thrombocytopenia (NAIT), who transitioned from frozen plasma (FP) infusions to home infusion of a virally inactivated plasma derived Factor VIII (vipdFVIII) concentrate with a high ADAMTS13 content.

Methods: A term female infant was noted to have petechiae, thrombocytopenia but no anemia. By Day 3, the platelet count was $9 \times 10^9 \text{ L}^{-1}$ so the child received platelet transfusions and intravenous immune globulin (IVIG) for NAIT confirmed by maternal antibodies and parental platelet typing. At 5 months old the patient was readmitted to hospital for anemia and thrombocytopenia. Investigations revealed hemoglobin 62 g L^{-1} , platelet count $14 \times 10^9 \text{ L}^{-1}$, reticulocyte count 18%, and LD 1145 U L^{-1} . Blood film showed schistocytes and thrombocytopenia. ADAMTS13 activity level of < 5% with no inhibitor and genetic analysis confirmed diagnosis of cTTP. FP infusions were needed every 2.5 weeks to maintain normal hematologic parameters. Despite no reactions, alternate therapies were explored due to treatment burden. At 18 months old, the patient began infusions every 2–3 days of high ADAMTS13 content vipdFVIII.

Results: Hematologic indices remained normal to near normal on vipdFVIII infusions. ADAMTS13 activity was 10% 48 h after pdFVIII infusion. Trough FVIII levels were mildly elevated but lowered when the interval changed to 2 times a week. The patient had no adverse reactions. After 1 year the child remains on vipdFVIII home infusions administered by parents 2 times a week with normal end-organ status and no adverse clinical events.

Conclusion: Our success with switching a cTTP patient to home infusion with a high ADAMTS13 content pdFVIII suggests this therapy should be explored as a primary prophylactic treatment for cTTP.

Disclosure of Interest: None declared.

PO564-TUE

Anti-idiotypic darpins restore ADAMTS13 activity in Baboons challenged with human monoclonal antibodies: first step towards an animal model for human acquired TTP

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Background: Previously we have shown that anti-idiotypic Designed Ankyrin Repeat molecules (DARPs) restored ADAMTS13 activity by neutralizing plasma-derived autoantibodies in the majority of acquired thrombotic thrombocytopenic purpura (aTTP) patients *in vitro* (AAI abstract, Schaller et al, J Immunol, 2013, 190, 68.14).

Aims: The next step is now to develop an *in vivo* animal aTTP model in which the therapeutic potential of our anti-idiotypic DARPs can be evaluated.

Methods: Increasing concentrations (100–500 nM) of an equimolar pool of four previously generated inhibitory monoclonal (mAbs) containing 1 of the 4 CDR3 motifs shared by two aTTP patients (Schaller et al, Blood, 2014) were added to *Papio* baboon plasma ($n = 2$) prior to assessing ADAMTS13 activity by the FRETS-VWF73 assay. The neutralizing capacity of the anti-idiotypic DARPs was assessed by pre-incubation of the mAb spiked baboon plasma with an equimolar pool of four anti-idiotypic DARPs (1000 nM) prior to measure the residual baboon ADAMTS13 activity by FRETS-VWF73 assay.

Results: Baboon ADAMTS13 activity was inhibited by the human mAbs by 75% (200 nM mAb) and 97% (500 nM mAb). No inhibition was observed with 100 nM mAbs. Pre-incubation of the human anti-ADAMTS13 Abs (200 nM or 400 nM) with a pool of four anti-idiotypic DARPs completely restored the observed neutralization of baboon ADAMTS13.

Conclusion: Baboon plasma challenged with human mAbs directed against the main ADAMTS13 epitope (spacer domain), which differs from previously reported mouse mAb directed against the metalloprotease, showed similar severe functional ADAMTS13 deficiency that was restored following administration of anti-idiotypic DARPs *in vitro*. This indicates that a baboon model is likely a clinically relevant approach to explore anti-idiotypic therapy for human aTTP patients.

Disclosure of Interest: None declared.

PO565-TUE

The laboratory profile that predicts favorable outcome after Eculizumab therapy in patients with clinical features compatible with atypical hemolytic uremic syndromeMian H¹, Chan AK^{2,3}, Lau KK⁴ and Chan HH^{1,3}¹Medicine; ²Pediatrics; ³TAARI, McMaster University, Hamilton, Canada; ⁴Pediatrics, Hong Kong University - Shenzhen Hospital, Shenzhen, China

Background: Eculizumab (C5Ab) is approved for the treatment of atypical hemolytic uremic syndrome (aHUS). Because patients presented with aHUS-like clinical features may not be readily differentiated from those with TTP, the current consensus suggests at least a 5-day trial of plasmapheresis (PLEX) before C5Ab treatment. Yet, once C5Ab is started, it lasts for a minimum of 4 weeks and further PLEX is relatively contraindicated. When patients do not respond well to C5Ab, it should prompt the clinicians to look for other differential diagnosis (DDx) so that the appropriate treatment will not be delayed.

Aims: To define the profile of laboratory parameters in patients with aHUS responding to C5Ab.

Methods: The literature was searched using OVID Medline and Embase databases for English-written, human studies published between Jan 1980 to Apr 2014 with keywords 'eculizumab' and 'atypical hemolytic uremic syndrome'. Apart from duplicated, irrelevant and review articles, the other excluded articles were those contained no information on laboratory parameters within the first 4 weeks of C5Ab treatment and those utilized nonstandard C5Ab dosage regimen. The data of 45 patients were found from 27 publications. Their demography, PLEX scheme and C5Ab dosing information were extracted along with the laboratory data.

Results: On average, patients received 11 PLEX sessions before C5Ab was initiated. Platelets was the earliest parameter to normalize at a mean of 10.4 day (median 7, range 1–42), followed by lactate dehydrogenase at 18.8 days (median 13, range 3–56). In contrast, renal function recovered at a more variable rate. It took an average of 31.8 days (median 30, range 4–84) for normalization of creatinine in the responders.

Conclusion: This study illustrates that patients with aHUS-like clinical features should be carefully reassessed after the 2nd weekly dose of C5Ab. If the increase in platelets is suboptimal, other DDx should be reconsidered so that appropriate treatment will not be delayed by the use of C5Ab.

Disclosure of Interest: None declared.

PO566-TUE

The clinical analysis of 58 thrombotic thrombocytopenic purpura patients

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare hematologic emergency with high mortality, which is characterized by thrombocytopenia, microangiopathic hemolytic anemia, neurologic abnormalities, fever, and renal impairment. The main treatment of TTP is immediate plasma exchange (PE), additional therapies include corticosteroids, cyclosporine, rituximab, and so on.

Aims: The aim of this study is to analyze the clinical data of the TTP patients in our center from 1998 to 2014 to guide the diagnosis and therapy of TTP in the future.

Methods: Patients conformed to the TTP diagnostic criteria are included in this study. We summarized their epidemiologic characteristics, pathogeny, clinical manifestations, laboratory measurements, therapeutic methods and prognosis. SPSS17.0 software was used for statistical analysis, and COX regression equation was used for multi-parameter analysis. $P < 0.05$ was considered to be a significant difference between the groups.

Results: There were 16 males and 42 females. The mean age was 38.18 ± 15.38 years. The levels of PLT, Hb, and LDH were $17.78 \pm 15.36 \times 10^9 \text{ L}^{-1}$, $65.15 \pm 18.83 \text{ g L}^{-1}$, and $1169 \pm 668 \text{ IU L}^{-1}$, respectively. The percent of peripheral blood red cell fragments ranged from 1.3 to ~31%. The effective rates of patients using glucocorticoids, PE + glucocorticoids, PE + glucocorticoids + immunosuppressive agents were 100% (1/1), 71.43% (25/35) and 100% (13/13), respectively. Nine refractory and/or relapsed patients received rituximab additionally, and the effective rate was 100% (9/9). The median times for PLT and LDH to reach the normal level were 7 days and 10 days. According to the COX regression analysis, aggravated neurologic abnormalities ($P = 0.003$) was the poor prognosis factor for TTP.

Conclusion: PE was still the first choice for TTP treatment, and rituximab was an effective therapy for refractory and relapsed patients. Aggravated neurologic abnormalities were the poor prognosis factor for TTP.

Disclosure of Interest: None declared.

PO567-TUE

Rituximab therapy as prophylaxis against thrombotic thrombocytopenic purpura: comparison of standard and reduced dose regimens

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Background: Acute antibody-mediated thrombotic thrombocytopenic purpura (TTP) is a thrombotic microangiopathy with high morbidity and mortality. Rituximab is highly effective when given as prophylaxis in patients deemed to be at high risk of acute TTP relapse, to prevent relapse, but the ideal dosing regimen is unknown.

Aims: To compare outcomes between TTP patients given rituximab prophylaxis at standard dose (375 mg m^{-2}) vs. reduced dose rituximab (200 mg).

Methods: Single-centre retrospective cohort study of patients in remission, at high risk of TTP relapse, in whom ADAMTS13 activity dropped from normal to $< 15\%$, given rituximab prophylaxis, between 2005 and 2014. Patient demographics, laboratory parameters, dose and frequency of rituximab were recorded. Outcome measures included re-normalisation of ADAMTS13, relapse rate, and treatment free survival (need for further rituximab prophylaxis). Comparison was made of disease free survival for patients given standard dose vs. reduced dose rituximab.

Results: Rituximab was given in 40 patient episodes, to 27 patients (21F, 6M). 36/40 (90%) patients had a documented reduction in ADAMTS13 level of $< 15\%$, median Hb 135 g L^{-1} ($81\text{--}168 \text{ g L}^{-1}$) and median platelet count $260 \times 10^9 \text{ L}^{-1}$ ($109\text{--}397 \times 10^9 \text{ L}^{-1}$). 18 episodes (13 patients) received standard dose and 22 episodes reduced dose rituximab. Normalisation of ADAMTS13 within 3 months, in 85% patients. Median treatment free survival was 30 months, with no significant difference between groups ($P = 0.3077$): 9/18 (50%) patients in standard dose group required re-treatment with rituximab (treatment free survival 34 months) vs. 9/22 (41%) patients in reduced dose group (treatment free survival 24 months). There were no acute TTP relapses in either group.

Conclusion: Rituximab therapy is effective as prophylaxis normalising ADAMTS13 and preventing acute TTP relapses in patients with immune TTP. There is a shorter, but non-significant time to re-treatment in the low dose rituximab group.

Disclosure of Interest: None declared.

PO568-TUE

Clinical and biochemical studies of a patient with acute thrombotic thrombocytopenic purpura (TTP) treated with N-acetylcysteine (NAC)

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Background: We previously found that NAC decreased von Willebrand factor (VWF) multimer size and function in human plasma and reduced microvascular thrombosis in ADAMTS13-deficient mice.

Aims: To evaluate the effect of NAC as adjunct therapy in a patient with relapsed TTP.

Methods: A 48-year-old female with 5 prior episodes of TTP complicated by myocardial infarction and stroke presented with acute TTP (ADAMTS 13 9%, positive inhibitor, platelet count $6000 \mu\text{L}^{-1}$, myocardial ischemia) and was treated with NAC per IRB-approved protocol [150 mg kg^{-1} bolus over 1 h and 9 mg $\text{kg}^{-1} \text{h}^{-1}$ as continuous infusion until the next therapeutic plasma exchange (TPE)]. She received daily TPE for 7 days, and intravenous NAC during days 2–5. Blood was collected daily for research assays.

Results: The patient's symptoms resolved and platelet count and LDH normalized by NAC day 3. Both parameters remained in the normal range after discontinuation of NAC and TPE. The free thiol concentration (NAC and cysteine, measured by mass spectrometry) in plasma peaked after NAC day 3, to ~5-fold the baseline concentration. This was accompanied by increasing ADAMTS13 activity from 9% at baseline to 94% on NAC day 3, the corresponding ADAMTS13 antigen levels being 49% and 39%, respectively. ADAMTS13 activity decreased to 66% after discontinuation of NAC. The VWF multimer size decreased during NAC and the VWF multimers migrated slightly faster. NAC also appeared to inhibit platelet activation. Before NAC infusion, the platelets were positive for phosphatidylserine (PS, 51%) and P-selectin (25%), compared to 2% and 5%, respectively, in a normal control. The percentages of PS- and P-selectin-positive platelets decreased to 18% and 10% respectively, during NAC treatment, but increased to 28% and 14% after discontinuation of NAC.

Conclusion: NAC treatment of a patient with TTP in conjunction with TPE was well tolerated and associated with increased ADAMTS13 activity, decreased VWF multimer size, reduced platelet activation, and recovery of platelet count and LDH.

Disclosure of Interest: None declared.

PO569-TUE

Autoantibody induced thrombotic thrombocytopenic purpura (TTP) in a young patient with metastasizing cancer of unknown primary (CUP) syndrome

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Background: Thrombotic microangiopathy (TMA) has with increasing frequency been reported in cancer patients. The characteristics suggest a different pathomechanism from classical TTP. Reports show subnormal, normal and very rarely severely deficient activity of ADAMTS13, usually with no detectable inhibitor.

Aims: We report the case of a 33-year-old woman with TTP and CUP syndrome with severely deficient ADAMTS13 activity and inhibitor.

Methods: The patient noticed a vulva lesion in 2009 and swollen inguinal lymph node in 2010 without further examination. In 2011 she was treated successfully with plasmapheresis (PP) and corticosteroids (C) for her first TTP bout. In 2013 she had a second bout after an infection. In 2014 she noticed a growing inguinal tumor which was diagnosed as squamous cell carcinoma metastasis from a cancer of unknown primary (CUP). Shortly after surgery a third TTP bout was diagnosed and treated with PP and C. The fourth bout was also diagnosed in 2014 after irradiation of the pelvis, complicated by urinary infection. A prolonged treatment with PP, C and rituximab was necessary to sustain complete remission. In 2015 two new pelvic lymph node metastases were diagnosed and chemotherapy recommended.

Results: In all TTP episodes our patient had ADAMTS13 activity < 5% and measurable inhibitors with the typical parameters of microangiopathic hemolysis and thrombocytopenia.

Conclusion: Most neoplasia associated TMAs do not have severe ADAMTS13 deficiency. Our patient had an autoimmune ADAMTS13 deficiency with recurrent acquired TTP episodes. Whether the acute disease was triggered also in the first two bouts through the not yet detected CUP remains unknown. We hypothesize that the surgical removal of the malignant lesion and the irradiation may have induced the onset of the acute disease (second hit) based on a preexisting autoimmune ADAMTS13 deficiency. We conclude that TTP patients should also be considered for cancer screening and 'neoplasia-associated TMA' does not exclude true autoimmune TTP.

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PO570-TUE

Severe maternal morbidity and fetal death in a pregnant woman with recurrent acquired thrombotic thrombocytopenic purpura (TTP)

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Background: Pregnancy is a very strong trigger for initial and recurrent bouts in hereditary TTP patients. The association with acquired TTP is less clear. In pregnancy, TTP must be distinguished from severe pre-eclampsia and hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome.

Aims: We report the case of a 39-year-old woman who presented in 09/2014 in week 18 of her second pregnancy with multiple hematomas in her lower limbs. During her first pregnancy she had suffered a first acquired TTP bout.

Methods: Now laboratory findings showed a second bout, she was immediately treated with corticosteroids (C) and plasmapheresis (PP). An ultrasound examination showed a fetus with adequate biometrics, normal placenta and low amniotic fluid. During the following days parameters deteriorated with renal failure, proteinuria, arterial hyper-

tension and edema. Therapeutic procedures were intensified with PP twice daily, acetylcysteine, rituximab (R) and antihypertensive medication. There was no fetal growth and a zero-flow of the umbilical artery. An interruption was performed in week 21. Postpartum severe hypertension, edema and renal failure persisted with several episodes of pulmonary edema. Dialysis was performed since 10/2014 and eculizumab was given 5 times once weekly.

Results: Eculizumab did not correct the severe renal failure but since 11/2014 platelets and hemolytic parameters improved and PP could be reduced. In 02/2015 the patient was still on dialysis, PP had been stopped for a few days.

Conclusion: Our patient suffered from a prolonged severe TTP episode with additional signs of preeclampsia, HELLP and atypical hemolytic uremic syndrome (aHUS), not improving under standard therapy and after pregnancy interruption. She had a severe ADAMTS13 deficiency with inhibitor titer during and before pregnancy but both of her TTP episodes were triggered by pregnancy like in hereditary TTP. ADAMTS13 and alternative complement pathway mutations are still pending and will be presented for further differentiation

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PO571-TUE

Obesity as a risk factor for the development of thrombotic thrombocytopenic purpura in ADAMTS13 deficient mice

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Background: Thrombotic thrombocytopenic purpura (TTP) is characterized by severe thrombocytopenia, hemolytic anemia and disseminated microvascular thrombosis leading to organ failure and death. Several factors like pregnancy, infections and surgery have been identified as risk factors for TTP. Recent studies have suggested that other factors like obesity might also induce TTP.

Aims: To investigate whether obesity is a risk factor for TTP using an *Adamts13*^{-/-} mouse model.

Methods: *Adamts13*^{-/-} mice were kept on a standard fat diet (SFD) or a high fat diet (HFD) for 20 weeks. Recombinant human von Willebrand factor (rhVWF) was injected to trigger TTP. Blood samples were taken after 24 h to determine blood platelet counts, hemoglobin levels, lactate dehydrogenase (LDH) levels and endogenous VWF levels.

Results: As expected, after 20 weeks, *Adamts13*^{-/-} mice on HFD gained more weight than *Adamts13*^{-/-} mice on SFD ($P < 0.0001$; $n = 18$). Interestingly however, obese mice had a lower platelet count ($894 \pm 35 \times 10^3$ platelets μL^{-1} , mean $\pm$ SEM) than lean mice ($1010 \pm 35 \times 10^3$ platelets μL^{-1} ; $P < 0.05$; $n = 38$) suggesting that obese mice might be more susceptible to TTP development than lean mice. Indeed, lean mice triggered with a threshold concentration of 250 U kg^{-1} rhVWF did not develop TTP, while typical TTP symptoms developed in obese mice. Obese mice had severe thrombocytopenia ($179 \pm 40 \times 10^3$ platelets μL^{-1}) and higher LDH levels ($703 \pm 62 \text{ mU mL}^{-1}$) compared to their lean counterparts ($498 \pm 49 \times 10^3$ platelets μL^{-1} ; $161 \pm 62 \text{ mU mL}^{-1}$ LDH; $P < 0.0005$; $n = 18$). No difference in hemoglobin levels were observed between the lean and obese mice after the administration of rhVWF. More endogenous VWF was released in the obese mice ($319 \pm 33\%$) compared to the lean mice ($192 \pm 20\%$) during the TTP episode ($P = 0.0056$; $n = 18$).

Conclusion: After HFD, *Adamts13*^{-/-} mice are more susceptible to TTP than mice on a SFD, indicating that obesity is a risk factor for the development of TTP in mice. Disclosure of Interest: None declared.

PO572-TUE

A multicenter, randomized study of cyclosporine or corticosteroids as an adjunct to plasma exchange in initial therapy of thrombotic thrombocytopenic purpura

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Background: A prospective, randomized study (NCT00713193) was undertaken to compare the efficacy and safety of cyclosporine (CSA) and prednisone (P) as adjuncts to plasma exchange therapy (PEX) in the treatment of acquired TTP.

Aims: The primary objective was to determine if CSA or P given as an adjunct to PEX has a lower exacerbation rate. Secondary endpoints included the effect of CSA and P on the ADAMTS13 (AD13) activity and the relationship to outcome.

Methods: Patients were randomized 1:1 to CSA ($2\text{--}3 \text{ mg kg}^{-1} \text{ day}^{-1}$ orally) or P ($1 \text{ mg kg}^{-1} \text{ day}^{-1}$ orally) with daily PEX until response was achieved (nml platelet, LDH). After stopping PEX CSA was continued for 6 months; P was continued for the first 4 weeks, then tapered over 4 weeks. Exacerbation was defined as recurrent thrombocytopenia requiring PEX < 30 days after the last PEX, with relapse defined as recurrence > 30 days after the last PEX. AD13 biomarkers were obtained pretreatment, weekly for 4 weeks after the last PEX, monthly for 5 months, and then every 3 months.

Results: Since November 2007, 26 patients were randomized to CSA ($n = 12$) or P ($n = 14$) and followed for a median 36 m (range 1–81). The study was halted for a planned interim analysis. Response rates were comparable in both arms (CSA: 11/12 [92%]; P: 11/14 [79%]; $P = 0.2$). The exacerbation rate on the CSA arm was 3/11 (27%) v. 1/11 (9%) on the P arm ($P = 0.3$). Pretreatment AD13 activity was equal on both arms (CSA: 2.8%, (range 0.5–7.4%); P: 1.7%, (range 0.5–12.4%); $P = 0.4$). After PEX the median AD13 activity was higher on P v. CSA each week during the first 4 wks, but was significantly higher only on week 3 (Wk 1: 18.4% v. 5.5%, $P = 0.2$; Wk 2: 26.0% v. 10.5%, $P = 0.5$; Wk 3: 53.6% v. 2.9%, $P = 0.004$; and Wk 4: 71.9% v. 3.9%, $P = 0.105$).

Conclusion: There was no significant difference in the exacerbation rate between the CSA and P arms. However, improvement in the AD13 activity was significantly better in the P arm. The AD13 data and trend toward more exacerbations in the CSA arm suggest that P is superior to CSA as an adjunct to PEX therapy.

Disclosure of Interest: None declared.

PO573-TUE

Comparing performances of complement activation biomarkers between responders and non-responders to eculizumab therapy

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Background: This is a pilot report from an ongoing study to define the role of complement biomarkers in managing patients receiving eculizumab therapy.

Aims: Defining the clinical utility of complement biomarkers in eculizumab therapy.

Methods: In the OSU-TMA (thrombotic microangiopathy) program, 26 patients received eculizumab therapy for a working diagnosis of aHUS. In this cohort, 24 patients had pre-treatment (Pre-T) samples while 19 had both pre- and serial post-treatment (post-T) samples for study. In this report, 146 serial samples were evaluated for C4d, C3a, C5a, C5b-9, and factor Bb and correlated with treatment outcome.

Results: Patients were followed for a mean of 23 mo (4 to 50) post eculizumab therapy. Based on both hematological and renal responses, 23 patients were grouped as responders (R) with either improved or stable kidney function without a later event of TMA. Three patients who showed either worsening renal function or persistent TMA while on eculizumab were classified as nonresponders (NR). In the pre-T samples, R group ($n = 21$) showed a more pronounced elevation in median values (ng mL^{-1}) of C3a (947 vs. 209), C5a (94 vs. 39), Bb (2632 vs. 1436), and C5b-9 (769 vs. 358) than NR group ($n = 3$). When evaluating the complement markers through the course of treatment, the post-T samples in R group ($n = 16$) exhibited a significantly lower C5b-9 level (310 vs. 872, $P < 0.01$) than the pre-T samples. In contrast, the level of C5b-9 in post-T samples in NR was only marginally reduced (263 vs. 358, $P = 0.36$). Median C5a levels were increased in both R and NR groups after treatment with eculizumab.

Conclusion: Patients who respond to eculizumab exhibit a more severe complement activation prior to the treatment. A significant drop of C5b-9 level after eculizumab therapy is associated with clinical efficacy. A future study using a larger cohort is needed in order to fully clarify the utility of complement biomarkers in the selection of patients receiving eculizumab and in monitoring the patients for clinical responses.

Disclosure of Interest: None declared.

PO574-TUE

Evaluation of long term neurological sequelae after a first episode of thrombotic thrombocytopenic purpura

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Background: Neurological involvement related to microthrombosis is the hallmark of acute thrombotic thrombocytopenic purpura (TTP); however, persisting neurological symptoms are often present in remission phase.

Aims: To assess the presence of neurological impairment in patients surviving the first episode of TTP and the overall impact on quality of life.

Methods: We performed a cohort study of consecutive patients referred to our Centre between 2007 and 2012 for a first episode of acute TTP. Patients who survived the acute event underwent a neuropsychological evaluation of memory and attentional functions with the standardized Neuropsychological Battery (ENB Profile) at least 6 months after the event. We evaluated short memory (word-list and prose memory), deferred and immediate memory, vigilance, divided and sustained attention. Health-related Quality of Life (HRQL) was also assessed by the European Quality of Life Five Dimension EQ-5D questionnaire. To investigate any changes over time, the same tests were repeated after at least 6 months. Scores obtained by the specific questionnaires were compared with published normative data, standardized by age and education level.

Results: Thirteen patients (8 females; median age 48 years, range 27–77) underwent the first evaluation after a median time of 21 months (range 5–78) from acute TTP. Of those, 11/13 (85%) had an abnormal neuropsychological evaluation. The domain of deferred memory was the most affected, with 8/13 (62%) patients reporting a pathological test. Attention appeared to be less affected, with divided attention being impaired in 4/13 (31%) patients. At the second evaluation, these results did not change. 11/13 (85%) patients reported moderate levels of anxiety/depression symptoms at the EQ-5D test at both evaluations.

Conclusion: In this pilot study, a large proportion of TTP patients demonstrated compromised memory functions and persisting anxiety/depression symptoms a few years after a single TTP episode.

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Venous Thromboembolism – Diagnosis II

PO576-TUE

Incorporation of right ventricular dysfunction into the simplified pulmonary embolism severity index as a predictor of complicated clinical course in low-risk patients presenting with acute symptomatic pulmonary embolism

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Background: In patients with acute symptomatic pulmonary embolism (PE), the simplified Pulmonary Embolism Severity Index (sPESI) identifies those at low risk for short-term PE-related complications.

Aims: We aimed to assess the benefit of adding transthoracic echocardiography (TTE) assessment of right ventricular (RV) dysfunction to the sPESI as another predictor of poor outcome.

Methods: We prospectively examined the cohort of patients with PE that enrolled in the multicenter PROTECT study. Using the baseline data collected at the time of PE diagnosis, the central coordinating center prospectively determined the simplified Pulmonary Embolism Severity Index (sPESI). Patients underwent transthoracic echocardiography (TTE) within 24 h after diagnosis of PE. This study used a *complicated course* (i.e., death from any cause, hemodynamic collapse, or adjudicated recurrent PE) as the primary endpoint, and follow-up occurred through 30 days after the PE diagnosis.

Results: of 848 patients, none had hypotension. Of the 37% (313/848) that had a low-risk sPESI score (34–40%), 5 (1.6%; 95% confidence interval [CI], 0.5–3.7%) experienced a complicated course that included 1 (0.3%, 95% CI, 0–1.8%) death. In this low-risk sPESI subgroup, 87% (273/313) did not show and 13% (40/313) did show RV dysfunction on TTE. Three (1.1%; 95% CI, 0.2–3.2%) of the 273 sPESI low-risk patients without RV dysfunction had a complicated course, and none died. The sPESI model had a sensitivity of 95% (95% CI, 86–99%) and a negative predictive value of 99% (95% CI, 97–100%).

Conclusion: In normotensive patients that had acute symptomatic PE, the sPESI accurately identified those at low-risk for a complicated course. Incorporation of RV dysfunction assessed by TTE into the sPESI did not significantly improve prognostication.

Disclosure of Interest: None declared.

PO577-TUE

Radiation and breast cancer risk in women diagnosed with pulmonary embolism: CT pulmonary angiography vs. ventilation-perfusion scanning

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Background: Computer tomography pulmonary angiography (CTPA) is a commonly used diagnostic test for pulmonary embolism (PE). However, there are concerns about greater radiation exposure and breast cancer (BrCa) risk in women undergoing CTPA vs. ventilation perfusion scanning (VPS).

Aims: We sought to determine the incidence of BrCa in women diagnosed with incident PE by CTPA vs. VPS.

Methods: Using the administrative healthcare databases of the Canadian province of Québec, we identified women with incident diagnosis of PE between 2000 and 2009 and without a history of BrCa. We excluded all women with 6 months or less of follow up following PE. We determined if CTPA or VPS scanning was used to diagnose PE. The main outcome was a new diagnosis of BrCa. Data is reported as mean (95% confidence intervals).

Results: In all, 12,515 women with incident PE fulfilled inclusion criteria. Overall, 2661 women had a CTPA, 4747 had a VPS, and 5107 had non-specified testing (NST). In all, 55 (2.07%) women with CTPA, 124 (2.60%) women with VPS, and 109 (2.13%) with NST were diagnosed with BrCa during a mean follow up of 3.68, 4.73, and 4.19 years, respectively. The incidence of BrCa among women aged < 45 years ($n = 2627$) tested by CTPA, VPS and NST was 3.04 (1.52–6.09), 1.37 (0.69–2.74), and 1.84 (0.92–3.67) per 1000 person-years, respectively. Among women 45 years of age and older ($n = 9888$), BrCa incidence following CTPA, VPS and NST was 6.57 (4.94–8.74), 6.99 (5.82–8.38), 5.92 (4.87–7.19) per 1000 person-years, respectively.

Conclusion: Our preliminary data suggest a higher incidence of BrCa with the use of CTPA (significantly higher radiation exposure to the breast) for diagnosing PE compared to VPS among women < 45 years of age. Further studies with larger sample size, longer follow-up and estimation of radiation dose received are needed.

Disclosure of Interest: None declared.

PO578-TUE

Risk-assessment model of recurrence within 3 months after a first episode of acute venous thromboembolism: Worcester venous thromboembolism study

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Background: Despite the proven benefits of anticoagulation, a systematic review indicated that the case-fatality rate of recurrent venous thromboembolism (VTE) is > 10% in the initial 3 months of acute treatment. Therefore, understanding who is at risk for developing VTE recurrence during the acute treatment phase may help clinicians determine the optimal frequency of clinical surveillance and appropriate outpatient treatment.

Aims: To develop a risk-score calculator to predict VTE recurrence during the initial 3-month acute treatment period.

Methods: Population-based surveillance study among residents of central Massachusetts (MA), USA, diagnosed with an acute first-time PE and/or lower-extremity DVT from 1999 to 2009 in hospital and ambulatory settings in all 12 central MA hospitals. Medical records were reviewed by trained abstractors and validated by clinicians.

Results: Among 2989 study patients, 141 developed recurrent VTE within 3 months of the index event. A risk-assessment model was developed using multivariable Cox proportional hazard regression (see Table). The predicted risks are 1.7%, 3.8%, 7.3%, and 15% for risk scores of 0, 1–12, 13–36, and 37+, respectively. A calculator based on patients without cancer will also be presented.

Predictor	Hazard ratio (95% CI)	Points
Hypercoagulable state	2.9 (1.3–3.2)	20
Inferior vena cava filter filter implanted	2.3 (1.6–3.4)	16
Varicose vein stripping	2.3 (1.2–4.2)	16
Major trauma	2.0 (1.2–3.2)	14
Taking anticoagulant at admission	1.9 (1.1–3.5)	13
Active cancer (with/without chemo)	1.7 (1.1–2.5)	11
Diagnosed with DVT alone at index visit	1.4 (1.0–2.0)	10
Possible maximum total risk score		100

Conclusion: Independent predictors were identified that may be useful for estimating risk of VTE recurrence for individual patients. The risk calculator can assist clinicians at the index encounter to determine the optimal frequency of clinical surveillance and appropriate outpatient treatment during the initial 3-month acute treatment phase.

Disclosure of Interest: None declared.

PO579-TUE

Performance of pulmonary embolism rule-out criteria (PERC) combined with low gestalt clinical probability in European chest pain population

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Background: The PERC rule is designed to rule out pulmonary embolism (PE) without further testing. In European population with high PE prevalence, its safety is currently not confirmed using alone or

combined with revised Geneva score. A recent study suggested good performances of the PERC rule when combined with a low gestalt clinical probability (CP).

Aims: To prospectively assess the performances of the combination of the PERC rule and low gestalt clinical probability in non-trauma chest pain population.

Methods: Prospective, observational study in two emergency departments. For each patient, clinicians were asked to assess gestalt CP for PE before any investigation: low, moderate or high. Among low CP patients, CP was considered as very low when clinician did not perform any test for PE diagnosis. PERC criteria were collected prospectively and PERC rule was calculated retrospectively. Main outcome was upper limit of 95% confidence interval (CI) for venous thromboembolism events (VTE) rate within 45 days.

Results: Among 628 enrolled patients, 11 had VTE (PE prevalence: 1.8%); 0/400 patients with a very low CP (0%), 4/197 patients with low CP (2%), 5/25 patients with moderate CP (20%) and 2/6 with high CP (33%). PERC rule was negative for 122 patients with very-low CP (30%): VTE false negative rate 0% [CI: 0–3.1] and, for 79 patients with low CP (40%): false negative rate 0% [CI: 0–4.6]. Ten computed tomographic pulmonary angiography and 75 D-dimer tests were performed in patients with a negative PERC rule and low CP.

Conclusion: In our population of chest pain patients, PERC rule combined with low gestalt clinical probability seems to safely exclude PE. The use of the PERC rule may decrease the number of further exams in this group of low CP patients, however PERC use instead of gestalt assessment would lead to overtest 70% of patients with very low CP. These results should be confirmed in a larger prospective study.

Disclosure of Interest: None declared.

PO580-TUE

An electronic tool and record for deep vein thrombosis (DVT) assessment, diagnosis, treatment and follow up

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Background: University Hospitals of Leicester acute ambulatory DVT service assesses 3000 patients a year and treats 600 new DVTs. Service improvements were required to optimise administrative processes. Our paper-based system was inappropriately diverting our DVT nursing team from delivering a very busy clinical service and making audit difficult.

Aims: We wanted to create an electronic DVT record within the same system as the computer aided anticoagulant dosing software. The vision of a paperless DVT module (assessment, diagnosis, treatment and follow up) with seamless referrals and communication with anticoagulation and primary care was born.

Methods: The whole DVT pathway has been sequentially computerised and tested within the clinical environment.

Results: The module first went live in August 2013. Between August 2013 and December 2014 we have assessed 4248 patients using the module; 675 (16%) were assessed as DVT unlikely with Wells score and d dimers and did not proceed to scan, 3426 had scans of which 754 DVTs (22%) and 147 cases of thrombophlebitis were diagnosed. Rivaroxaban became first line treatment for first DVTs in our service in October 2014 – a rivaroxaban treatment module captures the assessment, treatment and follow up of these patients – 100 to date. All primary care physician letters are generated within the system and sent electronically when possible.

Conclusion: Implementation of the DVT electronic tool and record has standardised and recorded each part of the patient pathway facilitating

audit, lean accountable practice and continuous improvement in workflow efficiency, quality and safety. The module also serves as a clinical database for benchmarking and research.

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PO581-TUE

Validation of the use of age adjusted d dimer cut off values to reduce the compression venous ultrasound rates in an acute ambulatory DVT service

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Background: As part of lean healthcare practice we wanted investigate ways of reducing unnecessary scans in our DVT service. The use of age adjusted D-dimer (AADd) cut off values ($\text{age} \times 10 \mu\text{g L}^{-1}$) has been reported to reduce the number of compression venous ultrasounds required in patients aged over 50 with a non-high clinical probability by improving Dd specificity without a significant effect on sensitivity.

Aims: To validate the utility of AADd in patients with a non-high clinical probability of DVT.

Methods: Consecutive patients with DVT unlikely scores using Wells 2 point testing had a quantitative near patient Dd. AADd cut off values were determined for all patients over 50. All patients with a Dd above the conventional Dd cut off of $500 \mu\text{g L}^{-1}$ proceeded to compression venous ultrasound.

Results: Over a 9 month period (January – September 2014) 714 consecutive patients with DVT unlikely Wells scores were identified. 510 (71%) were over 50 years old. 207/510 (41%) had a Dd $> 500 \mu\text{g L}^{-1}$ and proceeded to compression venous ultrasound imaging which was positive for DVT in 26 (13%). 83 of the 207 patients (40%) had a normal AADd and of these 82 had a negative compression venous ultrasounds, 1 had a small calf thrombosis. AADd cut off values increased the Dd specificity from 62% to 79%.

Conclusion: The use of AADd cut off increases the utility of Ddimer testing for the exclusion of DVT. It would safely reduce scans by 40% in patients aged over 50 with a non-high clinical probability of DVT. It would also increase the scan positivity rate in this group to 20%. Using age adjusted Dd cut off values would result in imaging examinations being correctly avoided in 77% of all patients with a low Wells score. This represents a significant saving in scan resource and reduction in patient waiting times.

Disclosure of Interest: None declared.

PO582-TUE

The use of an age-adjusted cut-off for D-dimer in screening patients for suspected DVT in the community can safely decrease the need for compression ultrasonography

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Background: D-dimer (DD) testing is used to rule out deep vein thrombosis (DVT) in patients with a non-high clinical probability score. DD concentrations rise with age and thus a higher proportion of older patients with suspected DVT may have DD concentrations >conventional cut-off. Thus a greater number of these patients may have unnecessary compression ultrasonography scans (CUS), as many will be normal. Previous studies have suggested that the use of an age-adjusted DD (AADD) in individuals of > 50 years can improve specificity without significant loss of sensitivity.

Aims: To determine whether the use of AADD in a primary care setting could safely decrease the number of CUSs required.

Methods: We carried out a 2-year retrospective analysis of patients > 50 years of age presenting with suspected DVT to a single community-based clinic. All patients were assessed using a Wells score and a quantitative Point of Care DD (cardiac DD). CUS was carried out as per standard diagnostic pathways. All patients discharged with DVT excluded had a 3-month follow (3 m-FU) up to determine whether they had developed a DVT during this time.

Results: Seven hundred and ninety-six patients were seen with 669 (84%) being > 50 years. 160/669 (20%) had a non-high Wells score but a DD above the conventional cut-off of 500 $\mu\text{g L}^{-1}$. 120 had CUS and 40 in whom DVT was clinically felt to be unlikely did not. There were 9 DVTs and 111 negative CUS. 3 m-FU in the CUS negative patients and those with no CUS showed 1 DVT in a patient with a negative CUS (0.7% failure rate). Of the 160 patients, 69 (43%) had an AADD below the cut off (age in years $\times 10 \mu\text{g L}^{-1}$). 33 had a negative CUS and 36 were not scanned as above. 66/69 had a 3-month follow up and none had developed a DVT.

Conclusion: Using an AADD cut off with a non-high Wells score can safely exclude a DVT in patients > 50 years and decrease the need for CUS by approximately 40%, an advantage in a population with a high percentage of older individuals with suspected DVT.

Disclosure of Interest: None declared.

PO583-TUE

Diagnostic prediction model vs. gestalt in the diagnosis of pulmonary embolism in primary care

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Background: Diagnostic prediction models, such as the Wells rule, can be used to rule-out pulmonary embolism (PE). However, there is conflicting evidence on the diagnostic value of a physicians' own unstructured probability estimate ('gestalt') as an alternative for these structured diagnostic models. Notably in primary care, the diagnostic value of gestalt for PE is unclear.

Aims: To compare the clinical performance of the Wells rule and gestalt, both scored by general practitioners, to rule-out PE.

Methods: We used the prospectively collected AMUSE-2 cohort suspected of acute PE ($n = 598$, prevalence 12%) by 300 general practitioners (GPs). GPs estimated the probability of PE presence in each suspected patient, on a scale from 0 to 100%, prior to calculating the Wells rule and point-of-care D-dimer testing (Clearview Simplify). PE was diagnosed by a composite reference standard of imaging techniques and follow-up. The low probability category for both diagnostic strategies was defined as 'Wells rule ≤ 4 and negative D-dimer' and 'GP-probability estimate < 20% and negative D-dimer'. Diagnostic accuracy (c-statistic), reclassification, efficiency (proportion low PE probability) and failure rate (proportion PE within low probability category) of the Wells rule and gestalt were compared.

Results: The median GP-estimated probability of PE patients was 0.70 (IQR 0.40), compared to 0.30 (IQR 0.32) in those without PE. The c-statistic was 0.80 (95%CI 0.75–0.86) for the Wells rule and 0.77 (95%CI 0.70–0.83) for gestalt. Whereas failure rates were comparable (Wells 1.5%, gestalt 1.3%), efficiency of the Wells rule was higher (45% vs. 25%). Three out of four false-negative patients when using Wells, were identified as high probability by gestalt.

Conclusion: The diagnostic prediction model and gestalt both can safely rule-out PE in primary care. However, GPs tend to overestimate PE probability: the referral rate of gestalt is high. Therefore, the diagnostic prediction model is considered to be more efficient in primary care.

Disclosure of Interest: None declared.

PO584-TUE

Clinical prediction rules plus D-dimer testing do not enable a safe exclusion of deep vein thrombosis or pulmonary embolism in elderly patients residing in nursing homes or homes for the elderly

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Background: Clinical prediction rules for suspected venous thrombosis – i.e. deep vein thrombosis (DVT) or pulmonary embolism (PE) – have never been validated in fragile multi-morbid elderly patients residing in nursing homes or homes for the elderly. Yet – when proven safe – their frailty and multi-morbidity makes preventing unneeded patient transfers to a secondary care facility highly desirable.

Aims: To determine if two clinical prediction rules (Oudega-rule for suspected DVT; Wells-rule for suspected PE) enable a safe and efficient exclusion of venous thrombosis in suspected elderly patients (aged 60 years or older) residing in nursing homes or homes for the elderly.

Methods: This was a prospective cohort study in Dutch nursing homes or homes of the elderly. Attending physicians scored the (respective) clinical prediction rule in each suspected patient. Low-risk patients – defined by a low score (Oudega ≤ 3 , Wells ≤ 4) plus a negative qualitative point-of-care D-dimer – were not referred. In all others referral was recommended. The presence of venous thrombosis was adjudicated by a composite reference based on available imaging tests plus 3-months follow-up. Main outcome was the proportion of venous thrombosis in the low-risk patients (failure rate), as well as the proportion of patients in whom referral could be withheld (efficiency).

Results: In total 642 patients were included (348 suspected DVT, 294 suspected PE). Venous thrombosis was confirmed in 247 (38%) patients (164 DVT, 83 PE). In patients suspected of DVT, 69 were identified as low-risk (efficiency 20%) and DVT was confirmed in 4

patients during follow-up (failure rate 5.8%, 95% CI 2.3–14). Conversely, for PE this was 85 low-risk patients (efficiency 29%), and confirmed PE in 5 (failure rate: 2.9%, 95% CI 0.8–10).

Conclusion: A clinical prediction rule plus D-dimer testing does not seem to be a safe tool to rule-out venous thrombosis in elderly patients residing in nursing homes or homes for the elderly.

Disclosure of Interest: None declared.

PO585-TUE

Combined assessment of thrombotic and haemorrhagic risk in acute medical patients

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Background: Acute medical patients have a high risk of venous thromboembolic events (VTE). Unfortunately, the fear of bleeding complications limits the use of antithrombotic prophylaxis in this setting. To stratify the VTE and haemorrhagic risk, two clinical scores (PADUA, IMPROVE) have been recently developed. However, it is not clear how many patients have a concomitant high VTE and haemorrhagic risk and what is the use of prophylaxis in this situation.

Aims: To assess the VTE and major bleeding risk in medical patients; to evaluate the use of antithrombotic prophylaxis in different classes of risk.

Methods: Patients admitted to internal medicine (January–December 2013) were prospectively included. VTE and haemorrhagic risk were evaluated in all the included patients. Use and type of anti-thrombotic prophylaxis was recorded.

Results: One thousand and seven hundred and sixty-one patients (mean age 77.6 years) were enrolled; 76.7% were at high VTE risk and 11.8% were at high haemorrhagic risk. Anti-thrombotic prophylaxis was used in 80.5% of patients at high VTE risk and in 6.5% at low VTE risk ($P < 0.001$), and in 16.6% at high haemorrhagic risk and in 72.5% at low haemorrhagic risk ($P < 0.001$). Prophylaxis was used in 20.4% at both high VTE and haemorrhagic risk and in 88.9% at high VTE risk but low haemorrhagic risk. At multivariate-analysis, use of prophylaxis appeared highly influenced by the VTE risk (OR 68.2, 95% CI 43.1, 108.0).

Conclusion: Many patients admitted to internal medicine were at high risk of VTE. Since almost 90% of them were at low hemorrhagic risk, pharmacological prophylaxis may be safely prescribed in most of these patients.

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PO586-TUE

Prediction of sepsis development in puerperas with systemic inflammatory response syndrome

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Background: The problem of predicting the development of sepsis is actively discussed in the modern literature.

Aims: To reveal of hemostatic prediction marker of risk of progression of sepsis in systemic inflammatory reaction in parturients.

Methods: We analyzed the postoperative period in 55 puerperas (age 19 to 43 years) with systemic inflammatory reaction syndrome (SIRS), treated in intensive care units of Krai Clinical Hospital #2 (Krasnodar, Russian Federation) in 2010–2014 years. Complex assessment of haemostasis include biochemical coagulation tests and thromboelastography. Statistical analysis include determination of relationship of registered indicators and progression of sepsis using Spearman's rank correlation and multivariate regression analysis.

Results: Depend of multivariate regression analysis, the most prognostically significant factor of risk of progression of sepsis in SIRS was thromboelastography MA parameter (the regression coefficient - $b = 0.652$, $P = 0.033$) and prothrombin time (the regression coefficient - $b = 0.275$, $P = 0.033$). In this study, the higher correlation ration and regression ratio revealed for thromboelastography MA parameter. This is logical because this parameter is integral for displaying of entire function of haemostas system. The formation of loose clot ($MA < 44$ mm), as summary consequence of both deficiency of coagulation factors, thrombocytopenia and compensatory hyperfibrinolysis, most clearly correlated with progression of sepsis in SIRS.

Conclusion: Thereby, thromboelastography is operative and convenient technique in prediction of progression of SIRS in sepsis, that may contribute to the early preemptive and goal-directed therapy of inflammatory complications. As well, the prothrombin time is prognostically valuable, which may reflect aggravation of deficiency of coagulation factors in progression of sepsis.

Disclosure of Interest: None declared.

PO587-TUE

Prospective validation of the simplified revised Geneva score

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Background: Pretest probability assessment by clinical decision rule (CDR) is an important step in the diagnostic management of patients with suspected pulmonary embolism (PE). However, a limitation to the use of CDR is that their constitutive variables and corresponding number of points are difficult to memorize. A simplified version of the revised Geneva score (i.e. attributing one point to each variable) has been proposed but never prospectively validated.

Aims: Prospective validation of the simplified revised Geneva score (sRGS)

Methods: We conducted a prospective management outcome study, the ADJUST-PE study, which primary aim was to validate an age-

adjusted D-dimer cut-off in patients with suspected PE. The sRGS was prospectively used to determine the pretest probability in a sub-sample of 1621 study patients.

Results: Overall, PE was confirmed in 294 (18.1%) patients. Using the sRGS, 608 (37.5%), 980 (60.5%), and 33 (2%) were classified as having a low, intermediate and high clinical probability, respectively. The corresponding prevalence of PE were 9.7%, 22.4% and 45.5%. There was no statistically significant difference in the proportion of patients classified in each category, nor in the prevalence of PE as compared with the RGS. Using the sRGS, 490 (30.3%) patients had a D-dimer test below $500 \mu\text{g L}^{-1}$ and 653 (40.4%) patients had a negative D-dimer test according to the age-adjusted cut-off (D-dimer levels < age per 10). Using the RGS, the figures were: 491 (30.3%) and 650 (40.2%). None of the patients having a low or intermediate clinical probability using the RGS or sRGS and a negative D-dimer, either according to the usual cut-off or to the age-adjusted cut-off, had a recurrent thromboembolic event during the 3-month follow-up.

Conclusion: The sRGS and the RGS have similar diagnostic accuracy and result in a similar yield and safety when used to exclude PE with the D-dimer test, including when using the age-adjusted D-dimer cut-off.

Disclosure of Interest: None declared.

PO588-TUE

Review of emergency department investigated DVT

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Background: Prior to the introduction of a new pathway for the investigation and management of acute VTE, we reviewed the patients investigated through the main trauma unit within our trust over a period of 12 months.

Aims: To highlight areas of poor compliance with quality standards and to focus on how these could be improved upon.

Methods: Using radiology codes, all dopplers ultrasound scans for 2013 were reviewed and those requested by the emergency department were scrutinised in detail and compared to the agreed NICE quality standards for VTE QS29. A total of 759 scans were reviewed, 55% of which were made via the emergency department.

Results: of the 418 patients referred for scan from the ED, 68% were referred directly by their primary care physician while 27% were self referred. 49% of patients had no documented Wells score, whilst 78% had a recorded d-dimer. Of those with a Wells score > 2, 76% had a d-dimer taken. 88 patients were reported as positive scan for thrombosis. 50 patients were scanned within the 24 h target as set by NICE, of which 30 were scanned within 4 h. 10 of those scanned within the 4 h received LMWH outwith NICE standard which advises anticoagulation only if unable to scan within the 4 h. 76% of those positive were referred to the outpatient VTE team, 100% of whom received compression hosiery within 3 weeks of diagnosis.

Conclusion: A review of the investigation has prompted the development of a direct access doppler service for primary care physicians to avoid unnecessary attendances at the ED. A standard proforma with the Wells score and when necessary the d-dimer will streamline the number of scans undertaken. All positive scans will be referred directly to the VTE team for appropriate onward management.

Disclosure of Interest: None declared.

Venous Thromboembolism – Epidemiology II

PO589-TUE

Prevalence of venous thromboembolism and related morbidity and mortality among hospitalized patients in Saudi Arabia (Savte Study)

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Background: VTE is fatal condition. Despite of its cost-effectiveness outcome prophylaxis is underutilized in many countries including KSA. VTE prevalence and incidence not defined in KSA.

Aims: The primary objectives to determine the percentage of VTE patients received antithrombotic prophylaxis. The secondary objective determine the mortality rate due VTE events, types of VTE events, the percentage of VTE patients in each ward type, the patients prescribed anti-coagulant therapy and adherence to it and the VTE mortality compared to hospital mortality

Methods: Seven hospitals in KSA have participated. During the period from 1 July 2009 till 30 June 2010, cases of VTE in the hospitals were collected using patients' medical records. Only patients with confirmed diagnosis of VTE included in the analysis

Results: One thousand and two hundred and forty-one of confirmed VTE included in the study. 21.4% and 78.6% of VTE in surgical and medical patients respectively. 40.9% of VTE cases received appropriate prophylaxis. 63.2% of surgical patients and 34.8% of medical patients received this prophylaxis ($P < 0.001$). Mortality rate was 14.3% of all patients representing 1.6% of total hospital mortality. Mortality was 13.5% for surgical patients vs. 14.5% for medical patients ($P > 0.05$). 89.4% of survived patients received anti-coagulation therapy at discharge and 71.7% of them were adherent to it

Conclusion: VTE is cause of morbidity and mortality in hospitalized patients. Prophylactic thrombolytic therapy is underutilized due to gap between guideline and practice for patients at-risk of VTE.

Disclosure of Interest: None declared.

PO590-TUE

Below knee deep vein thrombosis (BKDVT): a benign entity or not?

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Background: BKDVT is traditionally associated with less clinical sequelae such as thrombosis recurrence and malignancy, and often treated with shorter duration and lower intensity of anticoagulation.

Aims: Evaluate the characteristics of below knee DVTs in our study population to determine if the risk of recurrence and complications are similar to major VTE (above-knee DVT and pulmonary embolism).

Methods: Retrospective evaluation of consecutive BKDVTs from July 2011 to December 2012 at Austin and Northern Health, Melbourne, including demographics, provoking factors, associations and outcomes. Comparison was made to major VTE within this same cohort.

Results: Of a total of 1029 VTE cases, there were 279 (27%) episodes of BKDVT; 22% had concurrent PE. Median age was 63 years with male predominance (56% vs. 44%, $P = 0.003$). 96% was symptomatic. 46 patients (16.5%) had active malignancy with higher rates of concurrent PE (77% vs. 18%, $P = 0.0001$). 191 patients had isolated BKDVT without malignancy. Of these, 18% had a prior history of venous thromboembolism (VTE). Three (1.5%) were subsequently diagnosed with cancer, similar prevalence to those with major VTE (1.7%, $P = \text{NS}$). BKDVT were more likely to be provoked compared to major

VTE (72% vs. 55%, $P < 0.001$). Median duration of anticoagulation was 5.4 months vs. 7.0 months for major VTE. Patients with major VTE were more likely to experience grade III/IV bleeding complications (6.3% vs. 1.0%, $P = 0.003$) despite similar duration of therapy. Recurrence was similar to major VTE (6.8% vs. 8.7%, $P = 0.42$), with no difference between provoked and unprovoked BKDVT (7.7% vs. 9.3%). Mortality rate was 5.5% with no thrombosis-related deaths.

Conclusion: BKDVT has similar rates of recurrence and subsequent cancer detection compared to major VTE. Given the similar recurrence rate and proportionately less bleeding complications, investigation and treatment of BKDVT should not differ from major VTE. Further studies are required to determine the adequate length of anticoagulation.

Disclosure of Interest: None declared.

PO591-TUE

Etiology of portal venous thrombosis and budd-chiari syndrome in adults

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Background: Portal vein is formed by confluence of splenic-superior mesenteric veins. Budd-Chiari syndrome (BCS) is defined as hepatic venous outflow obstruction.

Aims: To investigate etiologies of chronic thrombosis of portal venous system (PVS) and BCS.

Methods: Patients from 2 centers were enrolled. Demographic data, comorbidities, surgery-drugs stories questioned. Causes of congenital thrombophilia, *JAK2V617F* mutation, levels of homocysteine, factor-VIII, fibrinogen were investigated. Levels of protein C, S, ATIII were not examined in patients with cirrhosis/in patients using warfarin.

Results: Seventy-three patients (33 men) were included. Median age was 46 years (18–82 years). There was BCS in 15, PVS thrombosis in 58 cases. Reasons predisposing to thrombosis were shown in table. A congenital factor was determined in 46.7% of BCS, 27.6% of PVS thrombosis ($P = 0.166$). No myeloproliferative disease determined in 3 *JAK2* mutants. Cavernous transformation was seen in 59.6% of PVS thrombosis; 26.5% of these had a congenital reason.

Underlying disease PVS/ BCS	Congenital PVS/ BCS	Acquired PVS/ BCS
Cirrhosis 31	Factor VLeiden 7 1	Surgery 24 8
Scleroderma 2	G20210A 5 2	Contraceptive 4
Behçet 1	ProteinC deficienc 6 4	Roaccutane 1
Ankylosing spondylitis 1	ProteinS deficienc 6 3	Etanercept 1
FMF 1	ATIII deficiency 3 3	Pregnancy 2
Rheumatoid arthritis 1		<i>JAK2</i> mutation 2 3
Myeloproliferative 3 1		Homocysteinemy 20 5
Malignancy 4		Elevated factor VIII 8 3
PNH 1 1		Elevated fibrinogen 4 2
Crohn's disease 1		
Nephrotic syndrome 1		

Conclusion: Underlying disease (mostly cirrhosis), gastrointestinal surgery are most common reasons. Reasons for congenital thrombophilia with other acquired etiologies increase risk. *JAK2* mutation alone is also a risk factor.

Disclosure of Interest: None declared.

PO592-TUE

Incidence of catheter-related venous thromboembolism events in acute leukemia patients; a retrospective study of the safety of peripherally-inserted central catheter

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Background: Central venous catheters (CVCs) are a leading cause of upper extremity deep vein thrombosis (UE DVT). There is little data on patients with acute leukemia (AL). Long term CVCs are required for chemotherapy in AL. Concomitant severe thrombocytopenia makes anticoagulation for CVC related thrombosis a challenge. Incidence of UE DVT has been reported to be increased in those with peripherally inserted central venous catheters (PICC) vs. those with centrally inserted lines.

Aims: Our objectives are to identify leukemia inpatients with a PICC and report the incidence of UE DVT.

Methods: We reviewed 161 charts for AL inpatients requiring a PICC line admitted to Hematology at the University of Alberta Hospital between 2003 and 2013. Baseline patient characteristics were recorded. All venous thromboembolic events were objectively confirmed on imaging studies. Incidence of catheter associated thrombosis was calculated.

Results: Three hundred and eleven patients were identified. We present the preliminary results of the first 161 reviewed charts. Of these, 126 met our inclusion criteria and 119 had at least one PICC line insertion. 107 (90%) had AML, 50 (42%) were smokers, 76 (64%) had cardiovascular risk factor, and only 9 (8%) had previous DVT. Overall, there were 236 PICC line insertions, with the 5FR dual lumen being the most commonly used PICC line (80%). Out of the 236 insertions, there were 28 (12%) new ipsilateral UE DVTs, 22 (79%) of which developed acutely (< 1 month), and 19 (68%) in thrombocytopenic patients (platelet < 50). Four (1.7%) concurrent VTEs were recorded. There was an incidence of 1.85 DVT per 1000 catheter days.

Conclusion: The incidence rate of PICC-related UE DVT in our AL patients is higher than predicted for a general cancer patient population. This data will be compared to a similar cohort of AL inpatients who received a centrally inserted CVC (Broviac/Hickman). Determining factors that are associated with a lower risk of DVT in this high bleeding risk population will be important to optimize patient care.

Disclosure of Interest: None declared.

PO593-TUE

Epidemiology of venous thromboembolism in the Framingham heart studyPuurunen MK¹, Gona P^{2,3}, Murabito JM⁴, Magnani JM⁴ and O'Donnell CJ⁵

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Background: Limited contemporary data exist on the epidemiology of venous thromboembolism (VTE), defined as deep venous thrombosis or pulmonary embolism, in the community. The contribution of traditional atherosclerotic risk factors towards VTE risk remains poorly established.

Aims: We sought to define the distribution of VTE subtypes, provide an estimate of VTE incidence, and assess the relations of traditional atherosclerotic risk factors with incident VTE in the Framingham Heart Study (FHS).

Methods: The ascertainment period was 1995–2012 in FHS, a prospective community-based cohort study of three generations. A physician endpoint committee identified and adjudicated incident VTE events by using clinical records and imaging results. FHS participants without VTE served as the referent cohort. VTE was classified as unprovoked, provoked, or cancer-related. We determined the incidence rates for VTE, and then used univariate and multivariable-adjusted Cox models to relate traditional atherosclerotic risk factors (age, sex, BMI, smoking, diabetes, hypercholesterolemia, hypertension) to VTE risk.

Results: The median follow-up time was 9.8 (Interquartile range 7.9–15.2) years. Among 9747 FHS participants there were 279 incident VTEs. Incidence of VTE across the whole study population was 26.8/10,000 person-years (95% confidence interval [CI] 23.8–30.1). 29.0% of VTEs were unprovoked, 40.1% provoked, and 30.8% cancer-related. After adjusting for atherosclerotic risk factors significant in univariate analysis, age (HR 1.66 per 10-year increase; 95% CI 1.51–1.84) and BMI > 30 (HR 1.78; CI 1.36–2.28) remained significantly associated with VTE risk in multivariable-adjusted analysis.

Conclusion: We report the contemporary incidence of VTE in a community-based cohort. Among traditional cardiovascular risk factors, age and obesity were significantly associated with increased VTE risk. Further research is warranted to identify novel preventable risk factors for VTE.

Disclosure of Interest: None declared.

PO594-TUE

Impact of incident stroke on future risk of venous thromboembolismRinde LB¹, Småbrekke B¹, Lind C^{1,2}, Njølstad I^{1,3}, Mathiesen EB^{1,3,4}, Hald EM^{1,2,5}, Brækkan SK^{1,2,5} and Hansen J-B^{1,2,5}

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Background: Growing evidence supports a bidirectional association between venous and arterial thromboembolism. Even though clinical data supports a relation between stroke and venous thromboembolism (VTE), the strength of the association remains to be settled at the population level.

Aims: To investigate the association between stroke and future VTE in a prospective cohort recruited from the general population.

Methods: Subjects from the Tromsø study ($n = 29,857$) without a previous history of VTE and stroke were followed from baseline (1994) to the date of a VTE-event, death or migration, or to the end of follow-up (2010). Cox regression models with age as time scale and stroke as a time-varying exposure were used to calculate hazard ratios (HR) adjusted for sex, BMI, diabetes, smoking, blood pressure, HDL-cholesterol, physical activity and education. The regional ethical committee approved the study and all subjects gave their informed written consent.

Results: There were 1515 incident strokes with 61 subsequent VTEs during a median follow-up of 15.7 years. The risk of VTE increased during the first 6 months after stroke (HR 10.95; 95% CI, 7.20–16.64), but declined rapidly thereafter and was no longer significant. The risk estimates were higher for deep vein thrombosis (DVT) (HR 13.41; 95% CI, 8.11–22.15) than for pulmonary embolism (HR 7.56; 95% CI, 3.56–16.28). Stroke displayed higher risk for provoked VTE (HR 16.84; 95% CI 10.29–27.58) and DVT (HR 18.57; 95% CI, 10.37–33.24) compared to unprovoked VTE (HR 5.30; 95% CI 2.33–12.04) and DVT (HR 7.05; 95% CI 2.56–19.43).

Conclusion: Incident stroke was associated with increased risk of VTE, and DVT in particular, during the first 6 months after the stroke event. The high risk estimates for provoked VTE in stroke patients suggest that additional predisposing factors, such as immobilization, potentiate the impact of stroke on VTE risk.

Disclosure of Interest: None declared.

PO595-TUE

Evaluating the accuracy of international classification of diseases 10th revision codes for venous thromboembolism (VTE) and major bleeding (MB) in emergency room (ER) dischargesAl-Ani F¹, Shariff S², Siqueira L¹ and Lazo-Langner A^{2,3,4}

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Background: The use of administrative databases to conduct epidemiological research in VTE has increased in recent years but relies on the coding accuracy for the diagnoses of interest. To date, validation stud-

ies of VTE and major bleeding in Canada have assessed coding systems no longer in use.

Aims: We sought to validate the accuracy of the International Classification of Diseases 10th Revision (ICD-10) codes for VTE and MB in ER discharges in a large hospital in Ontario, Canada.

Methods: We conducted a retrospective chart re-abstraction study at a university-associated teaching hospital in London, Ontario, Canada between July 2002 and March 2014. Included patients were adults with a VTE or MB code registered in the National Ambulatory Care Reporting System. VTE was defined using ICD-10 codes for deep venous thrombosis (DVT), and pulmonary embolism (PE), and MB was defined using codes for intracerebral hemorrhage (ICH), subarachnoid hemorrhage (SAH), upper gastrointestinal (UGIB), and lower gastrointestinal bleeding (LGIB). A random sample of 50 patients was selected for each group. Two blinded chart abstractors independently conducted data collection and adjudicated outcomes using a predetermined set of criteria.

Results: Three hundred patients (100 VTE, 200 MB) were included. Kappa statistics showed good agreement between the two chart reviewers for all groups. Overall, ICD-10 codes showed reasonable accuracy for identifying MB events with a PPV of 72% and a specificity of 78%. Diagnostic codes performed particularly well for SAH and ICH (PPV 88% and 90%, respectively). In contrast, codes for VTE had a moderate to poor accuracy with an overall PPV of 49% and a specificity of 66%.

Conclusion: Isolated ICD-10 codes for VTE had limited predictive ability for identifying DVT and PE in ER discharges in this study. In contrast, ICD-10 codes showed reasonable accuracy for identifying MB events and it would be reasonable to use them for research purposes.

Disclosure of Interest: None declared.

PO596-TUE

Asymptomatic central venous line – related thrombosis in children: feasibility of conducting a clinical study

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Background: Over 50% of thromboses in children are related to central venous lines (CVLs). Screening for CVL-related thrombosis is not routinely performed and hence the rate of asymptomatic CVL-related thrombosis is unknown. Determining the natural history of asymptomatic thrombosis is challenging.

Aims: To describe the feasibility of conducting ultrasounds (US), blinding results to the clinical team, for a study of asymptomatic CVL-related thrombosis in a paediatric intensive care unit (PICU).

Methods: Collaboration with the medical imaging team was crucial to the completion of the US in PICU. The medical imaging team was involved in the development of guidelines for the imaging procedure. The US were incorporated into daily workload of sonographers and where possible, US occurred at suitable time for the patient. A five day timeframe was set for US to be performed to accommodate sonographers changing workload and patients' clinical status. The researcher was in daily contact with sonographers and conducted information sessions with the PICU staff.

Results: Two hundred and five patients consented for the study. US were completed for 149 patients. The table below provides the reasons US were not performed.

US data collection	N = 205	%
US complete	149	72.7
US not performed	39	19
Ineligible after consent	17	8.3
Reasons US not performed	N = 39	
Dressing obstructed view of vessel	4	10.3
Not completed in timeframe/ prior to discharge	33	84.6
Parent refused	2	5.12

Conclusion: US is the gold standard for imaging and diagnosing internal jugular thrombosis; the most common site for CVLs in children. This study has demonstrated that performing blinded US in a clinical study is feasible. The timeframe for the US is a critical component to achieving feasibility. Such studies are crucial to understand the natural history of asymptomatic thrombosis in children, which in turn is crucial to enable proper risk benefit evaluation of any therapy.

Disclosure of Interest: None declared.

PO597-TUE

Cerebral venous thrombosis: a longitudinal follow up

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Background: Cerebral vein thrombosis (CVT) is an uncommon form of stroke. A prothrombotic risk factor or a direct cause is identified in 85% of patients; majority have a good neurologic outcome.

Aims: Our objective was to evaluate the demographic and clinical characteristics, risk factors, treatment modalities, and outcomes of patients with CVT.

Methods: We retrospectively reviewed the medical records of patients with CVT referred to our hematology clinic at a tertiary hospital from January 2006 to January 2015.

Results: We identified 24 patients: 18 (75%) were females, 15 (62.5%) Caucasian and mean age was 33 years. The most common presenting symptom was headache in 18 (75%) followed by grand-mal seizures in 5 (20.83%). The sites of thrombosis were transverse sinus thrombosis in 13 (54%), sagittal sinus thrombosis in 11 (46%), sigmoid sinus thrombosis in 6 (25%) and straight sinus thrombosis in 2 (8.3%). One third of patients had multiple risk factors for thrombosis. The most common risk factor was hyper-estrogen state, present in 13 (54%) of patients. Nine (37.5%) had at least 1 thrombophilia; heterozygous FIIG20210A polymorphism was the most common, seen in 4 patients (16.7%). Eighteen patients (75%) were treated with warfarin, 8 (33.3%) with low-molecular-weight heparin (LMWH), and 1 (4%) with Rivaroxaban. Sixteen (66.6%) patients received anticoagulation for a limited period of time (3 to 24 months, 5 (21%) patients remain on chronic anticoagulation, 1 patient is in the acute treatment period and 2 patients were lost to follow-up. 5 patients became pregnant and managed successfully with LMWH. Two-thirds of the patients had complete resolution of the thrombosis and 1/3 partial resolution. Only one patient had residual neurological sequelae.

Conclusion: In conclusion CVT is a serious and unusual type of venous thrombosis. Majority of patients have favorable outcomes with anticoagulation treatment. Duration of anticoagulation in these patients however, remains undefined.

Disclosure of Interest: None declared.

PO598-TUE

Pulmonary embolism: does the seasonal effect depend on age? A 10 years nationwide analysis of hospitalization and mortality

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Background: Circannual variations in the incidence and mortality of pulmonary embolism (PE) have been previously described. However, the influence of age on seasonal variation of incidence and mortality is not well established.

Aims: This nationwide study aimed to assess the influence of age on seasonal pattern in hospitalizations and mortality for PE.

Methods: The study used comprehensive records from the French hospital discharge databases between 2002 and 2012 and death certificates between 2000 and 2010. For each outcome and separately for each sex, monthly event counts were analyzed using quasi-Poisson models including a seasonal component and adjusting for the underlying time trend, age of patients and population size changes as an offset-term. Tensor-products of regression splines were employed to smooth together trend and age, and seasonal variation and age. The seasonal variations were modeled by cyclic cubic spline.

Results: During the analyzed periods, 578,203 patients with PE were hospitalized and 152,995 death certificates had a mention of PE. Compared with summer months, the winter peak was associated with 25% increasing rates in hospitalizations and mortality. The rates ratio of hospitalizations between the winter peak and the summer trough gradually increased with age. Indeed, the winter excess of incidence compared with summer linearly raised from 10% IC_{95%} [5–16] in 30-year-old men to 33% IC_{95%} [31–35] in 75 year-old men. In the same way, the incidences at the peak time was 13% IC_{95%} [9–18] and 34% IC_{95%} [31–36] higher in 30 and 75-year-old women respectively. In addition, peaks were shifted at the beginning of the winter for younger.

Conclusion: Our data support an influence of age on the PE seasonal variations with a more pronounced effect in older. More studies are needed to clarify underlying age dependent mechanisms. Sensibility to infections along with pronounced inflammatory state particularly in older could be a hypothesis.

Disclosure of Interest: None declared.

PO599-TUE

Implications of cytogenetics for venous thromboembolism in acute myeloid leukemia

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Background: Due to the high risk of thrombocytopenia and hemorrhage, thrombotic complications have received little attention in patients with acute myeloid leukemia (AML). Furthermore, the predictive role of cytogenetics on venous thromboembolism (VTE) has largely been ignored.

Aims: This study aimed to evaluate the incidence, risk factors, and prognostic aspects of VTE in AML.

Methods: A total of 811 consecutive patients with AML were enrolled and analyzed retrospectively. Cox time-dependent covariate regression analysis was used to identify the significant predictors of VTE develop-

ment. To minimize potential confounding factors, we used propensity-score matching to compare overall survival between patients with and without VTE.

Results: The 6-month and 1-year cumulative incidences of VTE were 3.1% (95% confidence interval [CI], 2.0 – 4.7) and 3.9% (95% CI, 2.6 – 5.7), respectively. Of the 26 cases of VTE, 22 (85%) developed within 6 months of leukemia diagnosis and 13 (50%) were catheter-related. In multivariate analysis, advanced age (≥ 65 years) (hazard ratio [HR], 2.70; $P = 0.03$) and increasing cytogenetic risk (common HR, 1.84; $P = 0.05$) were independent predictors of VTE. There was no significant association between VTE development and decreased survival ($P = 0.32$ for matched analysis).

Conclusion: Advanced age and increasing cytogenetic risk, well-known predictors for clinical outcome in AML, were also independent risk factors of VTE development. Our results suggest that VTE does not hold prognostic implications for AML.

Disclosure of Interest: None declared.

PO600-TUE

Long-term health-related quality of life after deep vein thrombosis is impaired compared to controls

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Background: Health-related Quality of Life (HRQoL) has been found to be affected after deep vein thrombosis (DVT). Whether the long-term HRQoL of these patients is impaired when compared to controls remains unknown.

Aims: 1- to assess long-term HRQoL after DVT compared to age- and sex-matched controls; 2- to determine predictors of impaired HRQoL after DVT.

Methods: Patients with DVT 1–10 years prior to study inclusion were identified from the thrombosis registry of Østfold Hospital, Norway, and invited to participate in this study. Patients completed the EQ-5D-3L questionnaire prior to study visit. Post-thrombotic syndrome (PTS) was evaluated by the Villalta scale. In order to recruit a control group, patients were asked to distribute the EQ-5D-3L questionnaire to 2 age- and sex-matched friends or relatives. The EQ VAS score was dichotomized into impaired and not impaired HRQoL, impaired HRQoL was defined as EQ VAS score below the 10th percentile among the controls. Predictors of impaired HRQoL were identified using multivariate logistic regression. Based on prior analyses, age, sex, recurrent venous thrombosis, PTS, unemployment and comorbidities were among the variables considered predictors for impaired HRQoL.

Results: A total of 250 patients (164 men) and 114 controls were included in the study. Mean observation time from DVT to inclusion was 5 ± 2 years. Mean age at inclusion was 56 ± 14 years. Patients scored significantly lower on all items in EQ-5D (mobility, self-care, usual activities, pain/discomfort and anxiety/depression) compared to controls. Mean EQ VAS score was also significantly lower among cases compared to controls, 81.9 (SD 14.6) and 71.9 (SD 18.6), respectively ($P < 0.001$). Only PTS (OR 5.5; 95% CI 2.3–13.4, $P = < 0.001$) and unemployment (OR 9.8; 95% CI 3.1–31.4, $P = < 0.001$) were found to predict impaired HRQoL.

Conclusion: Long-term HRQoL assessed with EQ-5D was significantly reduced in DVT patients compared to controls. PTS and unemployment were the only predictors of impaired HRQoL.

Disclosure of Interest: None declared.

PO601-TUE

Long-haul travel and the risk of recurrent venous thrombosisTimp J¹, le Cessie S^{1,2}, van Hylckama Vlieg A¹, Rosendaal FR^{1,3,4} and Cannegieter SC^{1,4}¹Department of Clinical Epidemiology; ²Department of Medical Statistics; ³Department of Thrombosis and Hemostasis;⁴Eindhoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands

Background: Long-haul travel has been shown to increase the risk of first venous thrombotic events 1.5 to 3-fold. However, since the absolute risk is low, temporary thromboprophylaxis is not justified to prevent a first thrombosis. The risk of thrombosis after long-haul travel in those with a history of venous thrombosis is not known, while thromboprophylaxis may be indicated in these patients.

Aims: We aimed to study the relation between long-haul travel (> 4 h) and recurrent venous thrombosis.

Methods: This study is a case-control study nested within a cohort of 4597 patients with a first venous thrombotic event who were followed over time for recurrence from 1999 to 2010 (MEGA follow-up study). At the end of follow-up participants reported via a questionnaire on risk factors for thrombosis, including episodes of long haul travel (> 4 h) in the first 3 months before a recurrent venous thrombotic event (cases) or in a random 3 month-period during follow-up for participants without recurrence (controls). In total 2723 (59%) participants returned the questionnaire. Odds ratios (OR), adjusted for age, sex and comorbidity at baseline, were calculated to compare risk of recurrence between groups with and without recent travel in a (1:1) matched analysis on time.

Results: The risk of recurrent venous thrombosis was not increased in participants with recent long-haul travel [OR 0.8 (95% CI 0.6–1.1)]. For a recent long-haul flight the OR was 0.9 (95% CI 0.6–1.4) while it was 0.8 (95% CI 0.5–1.1) for other types of long-haul travel (car, bus or train). These findings seem not readily explained by preventative measures taken during travel as these were equally distributed between cases and controls (all preventive measures during a flight: controls 69%, cases 71%; anticoagulant use: controls 23%, cases 29%).

Conclusion: Risk of thrombosis in patients with a history of venous thrombosis is not increased after long-haul travel, either by plane or by other types of travel.

Disclosure of Interest: None declared.

PO602-TUE

Recent discharge confers risk for venous thromboembolism

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Background: Hospitalized patients are at significant risk for venous thromboembolism (VTE) after discharge. Current guidelines do not recommend the continuation of VTE prophylaxis in the outpatient setting after hospital discharge. However, patients may benefit from pharmacologic prophylaxis to reduce VTE risk if a significant number of patients admitted with a VTE were recently discharged from the hospital.

Aims: The aim of this retrospective review was to determine the percent of patients admitted to the hospital with a primary diagnosis of VTE that were discharged in the prior 30 days. Other demographic factors such as age, length of stay, diagnosis and type of service were also determined.

Methods: All patients with a primary diagnosis of VTE and admitted to a large tertiary care academic medical center from 1/1/2012 to 9/30/

2014 were evaluated for discharge from the same institution within the past 30 days. It was assumed that those who had a primary diagnoses of VTE were admitted for this reason and had a new VTE on presentation.

Results: One thousand and hundred and eighty-two patients were admitted with a primary diagnosis of VTE as the reason for admission over this approximate three year period. 26.6% of these patients were recently discharged from the hospital in the prior 30 days. The most common diagnosis of the index hospitalization was cardiopulmonary disease. Age, length of stay and type of service were not predictive risk factors.

Conclusion: Hospitalization represents a significant risk factor for VTE. A large percentage of patients with an admitting diagnosis of VTE were recently discharged within the past 30 days. Further study is needed to determine which risk factors can be identified that would pose the greatest VTE risk allowing for a validated approach to patient identification for possible post discharge prophylaxis.

Disclosure of Interest: H. Shah Speaker Bureau of: Janssen, BMS, Pfizer, N. Christensen: None declared.

Venous Thromboembolism – Therapy II

PO603-TUE

A drug-drug interaction study of DS-1040 and aspirin in healthy subjectsDow J¹, Puri A², McPhillips P¹, Orihashi Y¹, Dishy V³ and Zhou J³¹Daiichi Sankyo Development Ltd., Gerrards Cross;²Hammersmith Medicines Research, London, UK; ³Daiichi Sankyo Pharma Development, Edison, USA

Background: DS-1040b is an inhibitor of the *activated form of thrombin-activatable fibrinolysis inhibitor* (TAFIa) under development for the treatment of thromboembolic diseases.

Aims: This was a Phase 1, open-label, single-dose study to assess the safety (including bleeding time) and tolerability of a single intravenous (IV) dose of DS-1040 after 5 days of aspirin treatment in healthy subjects.

Methods: A total of 18 healthy males and females of non-childbearing potential were enrolled in this study. Each subject received a loading dose of 300 mg aspirin on the morning of Day 1 followed by a daily dose of 75 mg aspirin on Days 2 to 5. On the morning of Day 5, the dose of aspirin was followed immediately by 6 mg DS-1040 treatment administered as 0.5 h IV infusion.

Results: A single IV dose of DS-1040 following 5 days of aspirin treatment was safe and well tolerated. Mean bleeding time increased throughout the aspirin dosing period from pre-treatment baseline to Day 4 (from 3.3 min to 9.1 min), however, there was no further increase in mean bleeding time following co-administration of DS-1040 with aspirin (8.2 min).

Conclusion: In healthy subjects, the co-administration of aspirin and DS-1040 was safe and did not result in further increase of bleeding time.

Disclosure of Interest: J. Dow Employee of: Daiichi Sankyo Development Ltd., A. Puri: None declared, P. McPhillips: None Declared, Y. Orihashi Employee of: Daiichi Sankyo Development Ltd., V. Dishy Employee of: Daiichi Sankyo Pharma Development, J. Zhou Employee of: Daiichi Sankyo Pharma Development.

PO604-TUE

Feasibility of comparing intermittent pneumatic compression vs. a novel device for prevention of venous thromboembolism in trauma patients with contraindication to anticoagulationBhagirath V¹, Al Onazi N², Schulman S¹ and Crowther M¹¹Department of Medicine; ²Department of Surgery, McMaster University, Hamilton, Canada

Background: There is a high incidence of venous thromboembolism (VTE) in trauma patients, yet many cannot receive anticoagulant VTE prophylaxis due to bleeding risk. Intermittent pneumatic compression devices (IPCs) are commonly used in these patients. A small, self-adhesive, battery powered device (the novel device) which increases venous flow by stimulation of the common peroneal nerve has been approved by Health Canada, but its performance for VTE prophylaxis has yet to be tested in a patient population.

Aims: To test the feasibility of performing a large, event-driven study comparing the novel device vs. IPCs in trauma patients with contraindication to anticoagulant VTE prophylaxis.

Methods: Adult patients hospitalized for trauma at the Hamilton General Hospital with contraindication to anticoagulation were eligible. Subjects were randomized to IPCs or the novel device. Compliance was measured by twice daily bedside visits. Compression ultrasound studies were mandated at study exit; venous and arterial flow were measured for studies performed on weekdays. A tolerability questionnaire was administered to non-intubated subjects. Informed consent was obtained from all subjects, and the study protocol was approved by the local research ethics board.

Results: Sixty-nine patients were identified for screening by the treating physicians over 12 months. The study met a pre-specified utility endpoint and was stopped after 19 enrolled patients. 11 subjects were randomized to IPCs and 8 to the novel device. One subject discontinued early due to lack of available IPCs, and was excluded from further analysis. All secondary outcomes were assessed per protocol for 72% of subjects. No subjects developed VTE, and no serious adverse events were detected.

Conclusion: A larger event-driven trial would not be feasible in this patient population due to slow recruitment. No safety, tolerability, or compliance concerns regarding the novel device were identified from this small study.

Disclosure of Interest: None declared.

PO605-TUE

Prediction of the bleeding risk during anticoagulation treatment for pulmonary thromboembolismZhai Z¹, Zhang Z², Yang Y¹ and Wang C¹¹Beijing Institute of Respiratory Medicine; ²Beijing Chao Yang Hospital, Beijing, China

Background: Bleeding, especially major and clinical relevant non-major bleeding is the most important complication of anticoagulation which might affect the prognosis of pulmonary thromboembolism. A score which can accurately determine the risk of bleeding may help to make decisions on anticoagulant use.

Aims: To assess the predictive ability of major bleeding in patients by using three stratifications which are OBRI score, RIETE score and SBRP score, and to develop a simple prediction model to estimate the clinical non-major bleeding risk within 3 months of anticoagulation therapy.

Methods: Consecutive patients diagnosed as the first episode of acute PTE who met the including criteria in Beijing Chao-yang Hospital from January 2009 to September 2013 were enrolled. All the patients were classified into three categories according to bleeding risk (low,

intermediate and high) in each score (OBRI score, RIETE score and SBRP score). The primary endpoint events were major bleeding and clinical non-major bleeding. The secondary endpoint events were recurrence of VTE and all-cause death.

Results: Five hundred and thirty-nine patients were included in our study. 16 (3.0%) had major bleeding and 73 (13.5%) had clinical non-major bleeding within 3 months of anticoagulation. (i) 3 months cumulative major bleeding was 3.0% (95% CI 0.01–0.05) and clinical non-major bleeding was 14.0% (95% CI 0.11–0.17) (ii) The areas under the ROC curve were 0.68 (95% CI 0.53–0.82) in OBRI score, 0.59 (95% CI 0.46–0.72) in SBRP score and 0.73 (95% CI 0.61–0.86) in RIETE score. (iii) A prediction model of clinical non-major bleeding risk was composed assigning 1 points to age ≥ 65 or diabetes, 1.5 point to cancer or rt-PA therapy.

Conclusion: The incidence of bleeding within 3 months of anticoagulation in our patients was high. A risk prediction model based on four variables documented at entry can stratify the risk of clinical non-major bleeding in PTE patients during the first 3 months of anticoagulant treatment.

Disclosure of Interest: None declared.

PO606-TUE

Recurrences after stopping anticoagulant therapy in Thai patients with non cancer-related venous thromboembolism

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Background: Preliminary data suggest a lower recurrent rate of venous thromboembolism (VTE) in Asian patients. In Caucasians, D-dimer levels at 1 month after stopping anticoagulant therapy of unprovoked VTE can predict recurrences. However, there is no report in Asians.

Aims: To determine the rate and risk factors of recurrent VTE in Thai patients.

Methods: This is a prospective cohort study. All adult patients with the first episode of non-cancer related VTE who had received 3 or 6 months of warfarin at King Chulalongkorn Memorial hospital between January 2013 and December 2014 were enrolled. The VIDAS D-dimer assay and thrombophilia tests (protein C, protein S, anti-thrombin and lupus anticoagulant) were performed at 4 weeks after warfarin discontinuation. Patients were followed for VTE recurrence and post-thrombotic syndrome (PTS) using the Villalta score.

Results: There were 42 subjects with the median age of 53.5 ± 32.5 years and 60.5% of them were female. The VTEs were unprovoked in 61.9%. Thirty (71.4%) cases were deep vein thrombosis (DVT) and 16.7% were isolated pulmonary embolism (PE). The durations of warfarin were 6 months in 60.9% with the median time in therapeutic INR of 59%. The high D-dimer levels (≥ 500 ng mL⁻¹) were detected in 54.8%. Contraceptive pill uses at the VTE onset was significantly associated with normal D-dimer after stopping anticoagulant ($P = 0.001$). Sixteen patients (38.1%) had PTS. After a median follow-up of 56 weeks, 5 patients (11.9%) had recurrent VTE. All of them had high D-dimer levels and PTS. VTE recurrences were associated with high D-dimer levels ($P = 0.04$) and PTS symptoms before recurrences ($P = 0.018$). The age, sex, presence of provoking factors and thrombophilia were not significantly associated with high D-dimer levels or recurrences.

Conclusion: VTE recurrences in Thai patients are not uncommon and associated with high D-dimer levels and prior PTS.

Disclosure of Interest: None declared.

PO607-TUE

XALIA, a non-interventional study comparing rivaroxaban with standard anticoagulation for initial and long-term therapy in deep vein thrombosis: baseline demographics and hospitalization rates

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Background: Rivaroxaban is approved for the treatment and secondary prevention of deep vein thrombosis (DVT) and pulmonary embolism (PE). XALIA is a multicentre, prospective, observational study of patients presenting with acute DVT. XALIA compares the safety and effectiveness of rivaroxaban with standard of care (SOC) in real-world practice.

Aims: To analyse baseline demographics and hospitalization rates for rivaroxaban and SOC groups in XALIA.

Methods: Data were collected on patients aged ≥ 18 years, with objectively confirmed, acute DVT treated with an anticoagulant for ≥ 3 months. The type, dose and duration of drug therapy were at the discretion of the attending physician.

Results: This analysis is based on 5096 patients enrolled in 21 countries; 2550 received rivaroxaban and 2545 received SOC (of whom most received low molecular weight heparin plus a vitamin K antagonist). Across both groups, 89.3% of patients had DVT alone; 10.7% also had PE at baseline. The most common risk factors for DVT at baseline were: recent hospitalization within 3 months (15.8%), cancer (10.7%) and thrombophilia (5.7%). Patients treated with rivaroxaban were younger than those in the SOC group (mean age 56.7 vs. 61.8 years), and the proportion of patients with creatinine clearance $< 50 \text{ mL min}^{-1}$ was lower (3.1% vs. 8.1%). Overall, 1850 patients were hospitalized; 685/2550 (26.9%) rivaroxaban-treated patients (DVT: 516; DVT+PE: 169) and 1164/2545 (45.7%) SOC patients (DVT: 873; DVT+PE: 291). Rivaroxaban patients had a shorter median hospital stay compared with SOC (5.0 vs. 7.0 days).

Conclusion: Patient characteristics such as age, comorbidities and hospital/medical guidelines influenced treatment allocation. This preliminary unadjusted analysis shows rivaroxaban-treated patients were less frequently hospitalized and had a shorter length of stay compared with SOC-treated patients.

Disclosure of Interest: A. Turpie Consultant for: Bayer HealthCare, Janssen Research & Development, Astellas, Portola and Takeda, L. Mantovani Grant/Research Support from: Boehringer Ingelheim, Pfizer, Bristol-Myers Squibb and Daiichi Sankyo, Consultant for: Bayer HealthCare, S. Haas Consultant for: Bayer HealthCare, Bristol-Myers Squibb, Boehringer Ingelheim, Daiichi Sankyo, Pfizer and Sanofi, R. Kreutz Consultant for: Bayer HealthCare, Berlin-Chemie, Bristol-Myers Squibb and Daiichi Sankyo, D. Monje Employee of: Bayer HealthCare, J. Schneider Employee of: Bayer HealthCare, W. Ageno Grant/Research Support from: Bayer HealthCare, GlaxoSmithKline, Alexion Pharmaceuticals and Boehringer Ingelheim, Consultant for: Bayer HealthCare, Bristol-Myers Squibb, Pfizer and Daiichi Sankyo.

PO608-TUE

Apixaban for treatment of venous thromboembolism in patients from study centres in Asia; a subgroup analysis of the amplify trial

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Background: In 5395 patients with acute venous thromboembolism (VTE), the AMPLIFY trial showed 6 months of oral apixaban was non-inferior to conventional therapy, with significantly less bleeding.

Aims: Anticoagulant-related bleeding may be more common in Asians because of lower body weights. This possibility was evaluated by comparing results from Asian and non-Asian study centres.

Methods: Patients received apixaban (10 mg BID for 7 days, then 5 mg BID) or enoxaparin (1 mg kg⁻¹ BID) followed by warfarin (INR 2 – 3). Clinical characteristics and rates of adjudicated major or clinically relevant non-major (CRNM) bleeding and recurrent VTE or VTE-related death in Asia and elsewhere were compared.

Results: of the 446 patients (8.3%) enrolled in Asia (246 in East Asia, mainly China, and 200 in India), 223 received apixaban and the remainder received conventional therapy. In Asian and non-Asian patients, mean ages were 52.2 and 57.4 years and mean body weights were 60 and 86 kg, respectively. Body weight was $\leq 60 \text{ kg}$ in 30% and 7% of Asian and non-Asian patients, and BMI was $\leq 28 \text{ kg m}^{-2}$ in 76% of Asian and 48% of non-Asian patients, respectively. In Asia, rates of major or CRNM bleeding with apixaban and conventional therapy were 3.1% and 6.7%, respectively (relative risk [RR] 0.47, 95% confidence interval [CI] 0.19–1.13). Elsewhere, these rates were 4.4% and 10.0%, respectively (RR 0.44, 95% CI 0.35–0.55). In Asia, rates of VTE or VTE-related death with apixaban and conventional therapy were 3.3% and 4.2%, respectively (RR 0.79, 95% CI 0.30 – 2.1). Elsewhere, these rates were 2.2% and 2.6%, respectively (RR 0.85, 95% CI 0.59 – 1.22).

Conclusion: Despite lower body weight and BMI, there was no evidence of more bleeding with apixaban or conventional therapy in Asia than elsewhere. Apixaban had similar efficacy relative to conventional therapy in Asian and non-Asian patients. Wide CI values due to limited sample size indicate the need for more data to confirm these findings.

Disclosure of Interest: A. Gallus Consultant for: Pfizer, G. Agnelli Consultant for: Pfizer, H. Buller Consultant for: Pfizer, A. Cohen Consultant for: Pfizer, T. Lee Employee of: Pfizer, R. Pak Employee of: Pfizer, G. Raskob Consultant for: Pfizer, H. Shi Employee of: Pfizer, J. Weitz Consultant for: Pfizer.

PO609-TUE

Should we use venous thromboembolism prophylaxis in patients with haematological malignancies receiving chemotherapy?Wiszniowski A¹, Szopiński P¹ and Warzocha K²¹Department of Vascular Surgery; ²Department of Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, Poland

Background: Patients with haematological neoplasms (acute myeloid leukaemia, AML; acute lymphoblastic leukaemia, ALL; chronic lymphocytic leukaemia, CLL; myeloma plasmacyticum, MP; Hodgkin lymphoma, HL; non-Hodgkin lymphoma, NHL) carry increased risk of venous thromboembolism (VTE). The mechanisms that link these malignancies to activated coagulation have not been fully identified. Additionally, a lot of haematological patients use portacath (venous port) or central venous catheter for chemotherapy.

Aims: The goal of this study was retrospective analysis of VTE prevalence and use of venous thromboembolism prophylaxis in patients with haematological malignancies receiving chemotherapy.

Methods: Within the period 2005–2013, the following patients were admitted to the hospital and subjected to chemotherapy: 327 ALL; 144 ALL; 491 CLL; 503 MP; 138 HL; 589 NHL. 58% of them had placed venous port or central venous catheter for chemotherapy. Only 12% of hospitalized haematological patients received venous thromboembolism prophylaxis low molecular weight heparin (LMWH) before chemotherapy. The clinical diagnosis of symptomatic VTE was confirmed by the objective imaging procedures. All patients with confirmed VTE were treated with LMWH at individual doses related to the platelet counts.

Results: VTE was diagnosed in 143/2192 (6.5%) patients during the first or subsequent hospitalization. 69% of them had placed central venous catheter devices. Any patient from this group did not receive venous thromboembolism prophylaxis before chemotherapy. During antithrombotic treatment, neither VTE recurrences, haemorrhagic complications nor heparin-induced thrombocytopenia occurred.

Conclusion: VTE is quite common complication in hospitalized patients with haematological malignancies receiving chemotherapy. Intensive effort at thromboembolism prophylaxis in these patients should be considered, especially, in patients with central venous access.

Disclosure of Interest: None declared.

PO610-TUE

Low-risk pulmonary embolism and length of hospital-stay: the Lorpelhs StudyDonadini MP¹, Castellana M², Gnerre P³, Regina ML⁴, Pomero F⁵, Re R⁶, Gallo A¹ and Dentali F¹ Ageno W¹ and Squizzato A¹¹Department of Clinical Medicine, University of Insubria, Varese;²Ospedale Evangelico Internazionale, Genova, Italy; ³Ospedale di Savona, Savona; ⁴Ospedale di La Spezia, La Spezia; ⁵Ospedale S. Croce e Carle, Cuneo; ⁶Ospedale Maggiore della Carità, Novara, Italy

Background: Patients with pulmonary embolism (PE) are commonly admitted to hospital for their initial treatment, though some of them may be suitable for a short-hospital stay. Validated tools are needed to assist clinicians in stratifying the risk of adverse events

Aims: Aim of this study was to retrospectively evaluate if length of hospital-stay varies according to PE risk of adverse events defined by the Pulmonary Embolism Severity Index (PESI) in daily clinical practice.

Methods: For each of the six participating Italian Centers, fifty consecutive adult patients with an objectively confirmed diagnosis of PE

between 2011 and 2013 were included. Patients were identified by direct chart review or by searching the administrative database of the hospital using discharges codes according to the 9th International Classification of Diseases. PESI was retrospectively calculated and the duration of hospital stay was recorded. Patients were treated according to local clinical practice.

Results: Two hundred and ninety-nine patients were diagnosed with PE within 72 h from hospital admission. Mean age was 73.5 ± 14.6 years, 55.2% were females. Median hospital stay was of 10 days (interquartile range [IQR] 7–14) with a mortality rate of 8.4% (25 patients). According to PESI score, 89 patients (29.8%) were classified at low risk (class I and II) and 210 (70.2%) at high risk (class III, IV, V), with a median hospital stay of 9 (IQR 6–12.5) and 11 (IQR 8–215) days, respectively ($P = .003$). Median hospital stay was 8.5 days for class I, 9 for class II, 10 for class III, 12 for class IV, 12 for class V ($P = .013$).

Conclusion: Overall, median hospital stay for PE was long. In this retrospective study, the difference of 2 days between PESI low-risk and high-risk classes is statistically, but not clinically significant. Whether adopting PESI in clinical practice can influence clinicians' decisions about early discharge needs to be prospectively evaluated

Disclosure of Interest: None declared.

PO611-TUE

Antithrombotic treatment and outcomes of non-malignant non-cirrhotic splanchnic vein thrombosis: a sub-analysis from the isth registryRiva N¹, Ageno W¹, Schulman S², Beyer-Westendorf J³, Grandone E⁴, Di Minno MND⁵, Sartori MT⁶, Malato A⁷, Martinelli I⁸, D'Amico EA⁹, Bucherini E¹⁰, De Stefano V¹¹, Di Nisio M¹², Poggio R¹³, Nardo B¹⁴, Winder A¹⁵, Farge-Bancel D¹⁶, Betti S¹¹, Dentali F¹ and IRSVT Study Investigators¹¹Department of Clinical and Experimental Medicine, University of Insubria, Varese, Italy; ²Department of Medicine, McMaster University, Hamilton, ON, Canada; ³Division of Angiology, Center for Vascular Medicine and Department of Medicine III, University Hospital 'Carl Gustav Carus', Dresden, Germany;⁴IRCCS Casa Sollievo della Sofferenza, S. Giovanni Rotondo;⁵Department of Clinical Medicine and Surgery, Federico II University, Naples; ⁶Clinical Medicine II, University Hospital of Padova, Padova; ⁷UO Ematologia con Trapianto, Policlinico Universitario of Palermo, Palermo; ⁸A. Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine and Medical Specialties, Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Milan, Italy; ⁹Hospital das Clínicas da Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil; ¹⁰Ospedale di Faenza, Faenza; ¹¹Institute of Hematology, Catholic University, Rome; ¹²Department of Internal Medicine, SS.ma Annunziata Hospital, Chieti Scalo;¹³Thrombosis Center, Galliera Hospital, Genoa; ¹⁴Department of Medicine I, Busto Arsizio Hospital, Busto Arsizio, Italy; ¹⁵Coagulation Unit, Wolfson Medical Center, Holon, Israel; ¹⁶Unité de Médecine Interne et Pathologie Vasculaire, Université Denis Diderot, Paris, France

Background: Splanchnic vein thrombosis (SVT) not associated with liver cirrhosis or solid cancer has been the focus of several small observational studies, which suggested that the bleeding risk may offset the benefit of the anticoagulant treatment.

Aims: To assess the outcomes of non-malignant non-cirrhotic SVT from an unselected cohort of SVT patients.

Methods: International prospective registry of consecutive SVT patients, enrolled from 2008 to 2012 and followed for 2 years. Thera-

peutic strategies and outcomes were analysed separately for the patients without solid cancer or liver cirrhosis. A Central Adjudication Committee classified outcomes as vascular events (venous or arterial thrombosis) or major bleeding (MB; ISTH definition plus the need for hospitalization).

Results: Three hundred and forty-seven of 604 patients had non-malignant non-cirrhotic SVT (median age 48 years, 59.7% males, 76.4% portal vein thrombosis). The most common risk factors were abdominal inflammation/infection (18.7%), myeloproliferative neoplasm (MPN) (14.1%), surgery (10.4%) and hormonal therapy (7.2%); in the remaining patients SVT was unprovoked. Fifty-one patients (14.7%) were not anticoagulated; 68 received parenteral anticoagulants alone (median duration 5.5 months, IQR 1.9–12) and 228 were started on vitamin K antagonists (median 24 months, IQR 11–24); 14 patients (4.0%) were lost to follow-up. The incidence of recurrent thrombotic events was 5.62/100 patient-years (pt-y) (95% CI, 4.01–7.86) and the incidence of MB was 1.82/100 pt-y (95% CI, 1.01–3.28). In multivariate analysis, anticoagulant treatment ($P < 0.001$) was associated with lower risk of vascular events, while MPN ($P = 0.005$) and unprovoked SVT ($P = 0.037$) were associated with higher risk. Anemia ($P = 0.102$) was associated with a trend to higher risk of MB, while anticoagulant treatment ($P = 0.126$) with lower.

Conclusion: The incidence of MB and recurrent thrombosis in non-malignant non-cirrhotic SVT is low. Anticoagulant treatment in these patients did not appear to increase the risk of bleeding.

Disclosure of Interest: N. Riva: None declared, W. Ageno: None declared, S. Schulman: None declared, J. Beyer-Westendorf: None declared, E. Grandone: None declared, M. N. D. Di Minno: None declared, M. T. Sartori: None declared, A. Malato: None declared, I. Martinelli: None declared, E. A. D'Amico: None declared, E. Bucherini: None declared, V. De Stefano: None declared, M. Di Nisio: None declared, R. Poggio: None declared, B. Nardo: None declared, A. Winder: None declared, D. Farge-Bancel: None declared, S. Betti: None declared, F. Dentali: None declared, IRSVT study investigators Grant/Research Support from: This study was supported by a grant from Pfizer, Canada, to ISTH. The authors have no relevant conflicts to declare in relation to this abstract.

PO613-TUE

A global prospective cohort study of dabigatran for the treatment of venous thromboembolism (re-covery)

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Background: Pivotal randomized double blind clinical trials of non-vitamin K antagonist oral anticoagulants vs. warfarin have been published on almost 30,000 patients with venous thromboembolism (VTE). However, less well studied is contemporary VTE management in routine clinical practice on a global scale. Therefore, we are launching a new non-interventional study, called RE-COVERY, to evaluate the management of acute VTE.

Aims: (i) To characterize patients with deep vein thrombosis (DVT) and/or pulmonary embolism (PE), (ii) to describe treatment patterns, and (iii) to assess the safety and effectiveness of dabigatran compared to vitamin K antagonists (VKAs) in routine care.

Methods: We will recruit patients from different countries in different continents. The study will be approved by ethics committees, and written informed patient consent will be obtained. To obtain data on contemporary global clinical practice, the study will evaluate baseline characteristics and management strategy on a broad population of patients with DVT and/or PE, regardless of how they are treated. Those patients receiving either dabigatran (110 mg or 150 mg twice daily) or VKAs will be followed up every 3 months for up to 1 year after enrollment. Overall, 6000 to 8000 patients will be recruited for RE-COVERY. Electronic case report forms and source data auditing will be conducted.

Results: RE-COVERY will begin in 2015, with final results expected in 2019.

Conclusion: RE-COVERY will characterize patients with VTE in routine care and, in conjunction with randomized trial data and observational data (e.g. from other registries), will enhance our understanding of how to improve and optimize VTE management.

Disclosure of Interest: S. Goldhaber Grant/Research Support from: Bristol-Myers Squibb, Boehringer Ingelheim, Consultant for: Boehringer Ingelheim, Merck, Bristol-Myers Squibb, Medscape, W. Ageno Consultant for: Boehringer Ingelheim, I. Casella Consultant for: Boehringer Ingelheim, C. K. Han Consultant for: Boehringer Ingelheim, G. Raskob Consultant for: Boehringer Ingelheim, S. Schellong Consultant for: Bayer Healthcare, Boehringer Ingelheim, GlaxoSmithKline, Sanofi, D. Singer Consultant for: Boehringer Ingelheim, D. Shash Employee of: Boehringer Ingelheim, M. Desch Employee of: Boehringer Ingelheim, S. Schulman Grant/Research Support from: Boehringer Ingelheim, Baxter, Octapharma.

Vitamin K Antagonists II

PO614-TUE

Influence of VKA-potentiating drugs on over-anticoagulation in atrial fibrillation

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Background: Vitamin K antagonists (VKAs) have been proven effective in patients with atrial fibrillation (AF), although a drawback is their sensitivity to drug-drug interactions. As a result, the altered dose-response relation can lead to suboptimal anticoagulation.

Aims: Given the limited data on this issue in daily practice, this study aims to investigate the association between the concurrent use of VKA-potentiating drugs and over-anticoagulation.

Methods: A prospective cohort of patients with AF using VKA for stroke prevention monitored by the outpatient anticoagulation clinic Nijmegen were contacted in May 2011 and followed until August 2012. Baseline characteristics, concomitant medication and INR measurements were recorded. Over-anticoagulation was defined as 1) the presence of INR measurements above 5.0 or 2) the time above an INR of 3.5 according to the Rosendaal method. We compared patients using drugs with a highly probable potentiating effect with patients using no VKA-interacting drugs.

Results: We included 1291 patients with a median age of 76 years (IQR 68–82) of whom 42% was female. Median follow-up was 1.2 years, comprising 32,660 INR measurements. No significant difference was observed for the presence of one or more INR's above 5.0, nor for the time above an INR of 3.5 (Table). Patients using potentiating drugs were more frequently monitored: 26 (IQR 20–34) vs. 24 (IQR 19–30) INR measurements, respectively ($P < 0.001$).

	VKA-potentiating drug use		
	Yes (n = 356)	No (n = 935)	P- value
<i>Over-anticoagulation</i>			
Patients with at least one INR measurement above 5.0	112 (31.5%)	265 (28.3%)	0.274
Patients with at least three INR measurements above 5.0	24 (6.7%)	39 (4.2%)	0.061
Median time above INR 3.5 (IQR)	16.7% (9.1–24.2)	17.6% (9.5–25.0)	0.066

Conclusion: In this real-world cohort study, the concomitant use of highly probable VKA-potentiating drugs was not significantly associated with over-anticoagulation. We did observe that patients with concomitant potentiating drug use were more intensively monitored.

Disclosure of Interest: None declared.

PO615-TUE

Agreement of point-of-care inr test with standard venous sampling assay at higher extreme ranges

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Background: Point-of-care (POC) coagulometers can potentially provide cost saving and convenience for patients and clinicians. In a previous study in 2007, it was demonstrated that the the CoaguChek[®] XS (Roche Diagnostics, Basel, Switzerland) can achieve high agreement with conventional venous sampling for International Normalised Ratio (INR) of < 3.5.

Aims: This study seeks to investigate if the same coagulometer is able to produce good correlation with venous INR even at extreme ranges of up to 5.0, and thus ascertain if it is also safe for clinical decisions to be made based on POC INR readings at the range between 3.5 and 5.0.

Methods: For every POC INR that is more than 3.5, a corresponding venous sample was drawn from the same patient. In total, 74 pairs of readings were obtained and analysed using STATA Version 13.1. The samples were stratified according to their POC INR results, of which 56 pairs fell between INR of 3.5 to 5 (Stratum A), and 18 pairs above 5 (Stratum B). Paired sample t-test was performed and Pearson Correlation Coefficient was computed in both strata.

Results: The magnitude of residual variation was observed to be smaller in Stratum A than Stratum B. In Stratum A, 66% and 84% of the POC INR readings fell within ± 0.5 and ± 0.8 of the standard assay results respectively. The correlation between POC and venous sampling method was found to be moderate ($r = 0.487$), with mean difference of 0.33 ± 0.49 ($P < 0.05$, 95% CI: 0.20–0.46). In Stratum B, 39% and 44% of the POC INR readings fell within ± 0.5 and ± 0.8 of the standard assay results respectively. The correlation between POC and standard assay was found to be poor ($r = 0.205$), with mean difference of 0.61 ± 1.22 ($P = 0.48$, 95% CI: 0.006–1.22).

Conclusion: The findings of this follow-up study re-affirms that the CoaguChek[®] XS Coagulometer is less robust in measuring INR at higher ranges, especially readings beyond 5.0. As such, caution should be exercised by clinicians when making clinical decision based on POC test results at extreme ranges.

Disclosure of Interest: None declared.

PO616-TUE

In anticoagulation treatment with vitamin K antagonists (AVKS): which is the best monitoring test?

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Background: In new anticoagulant's era (non-VKA oral anticoagulants - OACs) a lot of patients will continue benefiting with traditional treatment for different reasons, such as costs, renal impairment, etc. In this scenario it will be important to make our work better. But what is better?: Traditional International Normalized Ratio (INR) monitoring or Point of Care (POC) INR.

Aims: Compare two anticoagulation populations in the same Anticoagulation Clinic, with the same Medical Staff taking decisions. One population in POC monitoring and the other in Traditional Monitoring (TM), randomized by payment for one or the other methodology by their Medical Insurance

Methods: Retrospective analysis of 103 patients INR tested with POC technology (CoaguChek XSP[®] –Roche Diagnostics), and 103 patients with traditional test TCOAG TriniCLOT PT HTF human thromboplastine (ISI near 1.1) and Automated Autoanalyzer (Tcoag) Destiny Plus, from January 2014 to December 2014. Percentage of visit in range (Traditional INR %) and Time in Therapeutic Range (TTR) (Rosendaal Method for % INR in range – method which INR-specific person-time is calculated by incorporating the frequency of INR measurements and their actual values, and assuming that changes between consecutive INR measurements are linear over time) were calculated.

Results: TM populations (TMP) shows: TTR: 55.45% vs. POC population (POCP) TTR:62.57% (TMP: Total average days in range 156.02, Total average days of follow up 281.32; POCP 185.06 Total average days in range Total average days of follow up 295.78) ($P = 0.006$) and TMP % visit in range 52.85% vs. POCP 62.07% visit in range ($P = 0.001$).

Conclusion: Probably POC technology (reducing preanalytic and analytic variables) could be an alternative to improve INR test quality, making safer and comfortable traditional treatment with vitamin K antagonist. We think it's a valid alternative in all circumstances where OACs can't be used.

Disclosure of Interest: None declared.

PO617-TUE

Comparison of risk factors for warfarin-associated bleeding

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Background: Warfarin is the most commonly used oral agent for long term anticoagulation. Nevertheless, bleeding is the most frequent side effect of warfarin, thereby restricting its use. Risk factors for bleeding include age (65 and over), concomitant diseases, use of medicines, previous history of stroke or bleeding.

Aims: We aimed to evaluate the risk of bleeding in patients with warfarin associated bleeding and in patients under warfarin without bleeding.

Methods: 100 patients with bleeding under warfarin and 101 patients who has been monitored for international normalization ratio in our outpatient clinic were enrolled in the study. Data were statistically analyzed.

Results: There was no difference between two groups regarding age, gender, comorbidities and medications. The prevalence of bleeding was observed to be 2.8 fold more in patients who are 65 years or over while 5.6 fold more in patients with concomitant disease. History of

stroke was observed to increase major bleeding 3.2 fold. In the bleeding under warfarin group, 5 patients (5%) had INR levels 2.0–3.0, while 15 (15%) had 3.01–5.0, 32 (32%) had 5.01–10 and 43 (43%) had over 10. In the nonbleeding group 48 patients (48%) had levels of INR (< 2.0), 40 (39%) had 2.0–3.0 and 11 (11%) had INR levels 3.01–5.0. We have also observed that lack of education for medications may be a risk factor and patients may be under risk in the first 3-months' of medication. Regular monitoring of international normalization ratio in a certain center may decrease the risk while bleeding was more frequently observed in patients who have been followed up by cardiologists.

Conclusion: Older patients under warfarin should be educated and monitored on a regular basis for uncalled-for high INR levels and related complications. Patients taking warfarin and physicians prescribing warfarin should be cautious about these issues until new oral anticoagulants without restrictions of warfarin are developed.

Disclosure of Interest: None declared.

PO618-TUE

Expanding telemedicine to medical homes for comprehensive care delivery for persons with hemostatic disorders: a pilot study of the American thrombosis and hemostasis network (ATHN)/national hemophilia program coordinating center (NHPCC)

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Background: Telemedicine (TM) is the exchange of medical information from one site to another via electronic communications to improve patients' health status. Limited data exists about TM usage by hemophilia treatment center to deliver comprehensive care, to remote pediatric patients with hemostatic disorder.

Aims: To assess feasibility of telemedicine between specialists and medical home for children with hemostatic disorders, acceptability by patients, families, primary care physicians, primary care staff, specialist physicians, and specialist staff and cost of telemedicine visits vs. traditional visits.

Methods: TM site was the pediatrician's office at the Upper Great Lakes Family Health Center in Hancock/Houghton, MI, and 500 miles from Michigan State University (MSU) Center for Bleeding and Clotting Disorders (MSU CBCD). Vido™, a synchronous HIPAA regulated bidirectional videoconferencing technology was used. Records of patient visits diagnosis, travel distance time and costs, and interviews with physician staff and families were obtained. Measurable objectives included feasibility, acceptability and cost of TM.

Results: Beginning June 2014, a total of 13 visits by 10 patients, ages 2 weeks–16 years and their families were seen via monthly TM. Besides bleeding disorders, such as epistaxis, menorrhagia, von Willebrand's disease etc., other hematologic disorders were also seen. Eleven family members were tested. Patients incurred considerable cost savings by obtaining care locally instead of flying (cost \$1770) or driving (cost \$1158) to MSU CBCD. The MSU CBCD team also incurred significant cost savings. Patients, families and providers expressed satisfaction with the services. Besides education of medical home providers, technology issues were also addressed.

Conclusion: TM with medical home in remote areas is feasible and acceptable. Besides satisfaction with the services and there was considerable cost savings for both patients as well as the comprehensive care treatment team.

Disclosure of Interest: None declared.

PO619-TUE

Anticoagulation reversal in warfarin-associated intracerebral hemorrhage: a systematic review

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Background: Guidelines recommend therapy 'to replace vitamin K-dependent factors and correct the INR' in warfarin-associated intracerebral hemorrhage (WA-ICH). These guidelines are based on limited data and the effect of this intervention on functional outcomes is unknown. **Aims:** To systematically review published studies on warfarin reversal in WA-ICH and assess clinical outcomes

Methods: We used the MESH terms anticoagulant, warfarin, intracranial hemorrhage, and cerebral hemorrhage on Medline to search for articles through March 31, 2013. Of 845 articles identified, 134 abstracts were selected by two independent MD reviewers, and 59 articles for full review. Bibliographies were additionally searched and yielded one additional study. Thirteen studies met criteria for inclusion.

Results: All studies were retrospective and observational in design, and 11 were from a single center. There were no randomized trials. The reversal agents used varied and included fresh frozen plasma, vitamin K, recombinant factor VIIa, and prothrombin complex concentrate. Doses administered and times to intervention were not uniform. Nine of the 13 studies enrolled fewer than 60 patients, and 8 studies included functional outcome as an endpoint. Definitions of eligibility, disability, and time points for assessment of both functional outcome and mortality varied across studies. When reported, nearly one third of patients were excluded due to comatose state upon arrival. No reversal agent was shown to consistently improve neurological outcomes.

Conclusion: Despite availability of warfarin reversal agents, WA-ICH remains a highly fatal adverse event. Late patient presentation and time to treatment are contributing factors. Confounding by treatment, small sample sizes, and differences in inclusion criteria, dose, timing, and endpoint definition limit summary interpretation of published data. Given the high morbidity and mortality, prevention through risk modification remains critical in reducing this warfarin-related complication.

Disclosure of Interest: D. Ko: None declared, Z. Razouki: None declared, E. Hylek Consultant for: Bayer, Boehringer Ingelheim, Bristol Myers Squibb, Daiichi Sankyo, Janssen, Pfizer, Roche, and Medtronic.

PO620-TUE

The QUA-VKA study to identify new risk factors for bleeding during treatment with vitamin K antagonists: objectives and design

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Background: Patients who use vitamin K antagonists (VKA) are at increased risk of bleeding. Reported prediction scores for major bleeds perform modestly, necessitating the need for new predictors in this patient group.

Aims: The QUA-VKA prospective cohort study aims to identify new risk factors that contribute to the prediction of bleedings in patients who start VKA treatment. Here, we delineate the objectives of the study, principal methodological features, and timeline.

Methods: All patients starting VKA treatment at three Dutch anticoagulation clinics between January 1st, 2012 and July 1st, 2014 were included according to an opt-out procedure that was approved by the local medical ethical committee. Leftover plasma following analysis of the international normalized ratio was stored, and patients were followed until a major bleed, end of VKA treatment, death, or the end of the study (31 December 2014), whichever occurred first.

Results: Of 16,278 consecutive patients who started VKA treatment, 136 (1%) patients opted out, leaving 16,142 included patients. We obtained plasma from 13,528 patients (84%) as logistic complications prevented plasma collection for 2614 (16%) patients. 8545 Patients were male (52%), with a mean age of 70 years (SD 14) and the INR target range was 2.5–3.5 in 14,807 patients (91%). Preliminary results from two anticoagulation clinics showed that 306 major bleeds occurred during 15,188 years of follow-up (incidence rate 2.02/100 person years, 95% CI 1.80–2.25), of which 121 (incidence rate 3.84/100 person years, 95% CI 3.20–4.57) and 156 (incidence rate 2.66/100 person years, 95% CI 2.26–3.10) occurred during the first 3 and 6 months of VKA treatment respectively.

Conclusion: Long-term biological sample storage will allow for the investigation of biomarkers that may predict bleeding and other diseases. The coagulation factors VIII and von Willebrand factor are currently analyzed, and their association with major bleeds during VKA treatment will be available at the time of the ISTH2015.

Disclosure of Interest: None declared.

PO621-TUE

Assessment of the time in therapeutic range in patients with atrial fibrillation treated with vitamin K antagonists by specialists in haemostasis in Argentina: the TERRA registry

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Background: Vitamin K antagonists (VKA) should be monitored by the time in therapeutic range (TTR) which evaluates the percent of time that a patient remains in the targeted INR. In non valvular atrial fibrillation (NVAF) a low TTR (< 60%) has been associated with increasing risk of stroke, bleeding and death. Although some Latin America registries showed a low TTR (44%) in the region, there is no data available about the quality of anticoagulation in our country.

Aims: To assess the TTR in a multicenter network of patients with NVAF treated with VKA in Argentina.

Methods: The TERRA trial is a multicenter, observational study involving 14 anticoagulation clinics. Both private and public institutions recruited prospectively the first 100 consecutive patients with NVAF treated with VKA for at least 1 year. The INR was retrieved from controls and TTR calculated (Rosendaal method). The first 3 months of treatment with VKA were excluded from the analysis.

Results: One thousand and hundred and ninety patients were included in the analysis. Mean age was 75 ± 9.9 years, 52% were male. Mean TTR was 66.6 ± 19.4% (median 67.5%, IQI 54–80). Inter institution variability was marked, range 58 ± 17% to 88 ± 17%, $P < 0.001$. In all, 34% of the patients had a TTR < 60% and 20% a TTR < 50%. Age > 80 years, female sex and use of acenocumarol did not correlate with a low TTR. If the targeted INR would have been between 1.9 and 3.1, median TTR would have been 74.5%.

Conclusion: (i) Mean TTR in patients with AF treated with VKA in this multicenter network of Argentinean Haemostasis specialists was high and similar to some recent international therapeutic clinical trials (55–65%), indicating that high-quality anticoagulation with VKAs is possible in Latin America. (ii) Even with this level of excellence one

third of patients exhibited a TTR below 60% and might be candidates for an alternative management. (iii) The addition of TTR to clinical practice may help to improve the results of oral anticoagulation in patients with AF.

Disclosure of Interest: None declared.

PO622-TUE

Results from the prospective 'Farmamico' study: INR variations in warfarin-treated patients undergoing analgesic and anti-inflammatory therapies

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Background: FARMAMICO is a prospective study of pharmacovigilance involving 7 Italian centers, aimed to evaluate all interactions of oral anticoagulant therapy with other drugs and remedies.

Aims: In a sub-group of patients on warfarin, we evaluated the association between INR variations or adverse events with the use of analgesics or non-steroidal anti-inflammatory drugs (NSAID).

Methods: Two thousand and three hundred and seventy patients (1415 cases and 955 controls) from 2 centers (Bergamo and Brescia) from July 2009 to September 2014 entered the study. After adding a new medication, we defined as case an INR variation ≥ 1 above or below the target range and as a control an INR variation < 1. Patients filled self-reporting questionnaires on concomitant drugs. To investigate the association between analgesics (i.e. paracetamol) or NSAID (i.e. ibuprofen) and the INR variation or adverse event, the Relative Risk (RR) with 95% confidence intervals (95% CI) was estimated, first crudely and then adjusted for age and sex.

Results: 9.2% (130/1415) of patients on concomitant analgesics or NSAID showed ≥ 1 INR variations. Among them 70.7% had an INR above the therapeutic range, with a 15.4% of them with INR > 6. A 5.4% of total patients had instead an INR reduction. Analysis according to the drug classes showed that 56.3% of patients taking paracetamol had a ≥ 1 INR variations compared to 20.2% of patients taking ibuprofen. These latter had a 5-fold lower risk of INR variation compared to patients taking paracetamol [RR: 0.2 (95% CI: 0.11–0.36)]. This result is statistically significant and persists after adjustment for age and sex. Only one patient, during paracetamol, reported an adverse event (minor bleeding).

Conclusion: Our analysis show that paracetamol, considered the first choice analgesic for anticoagulated patients, enhances the anticoagulant effect of warfarin, while the anti-inflammatory drug ibuprofen seems to be safer in these subjects. It is advisable INR monitoring after few days from paracetamol introduction and for the first 2 weeks.

Disclosure of Interest: None declared.

PO623-TUE

Use of proton pump inhibitors among anticoagulated patients: the Michigan anticoagulation quality improvement initiative (MAQI2) experience

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Background: A multi-society (ACC/AHA/ACG) consensus group has recommended the use of proton pump inhibitors (PPIs) in anticoagulated patients at increased risk for gastrointestinal bleeding (GIB).

Aims: To assess the prevalence and predictors of PPI use among new warfarin patients.

Methods: We performed a retrospective analysis of new warfarin patients referred to 7 anticoagulation clinics in Michigan, USA, from 10/2009 to 12/2014 as part of the IRB approved Blue Cross Blue Shield of Michigan-sponsored Michigan Anticoagulation Quality Improvement Initiative. Medications and labs were collected at clinic enrollment. Unadjusted rates of PPI use were calculated, with stratification by risk category. Logistic regression and Cochran-Armitage Trend tests were used to assess predictors and trends in PPI use.

Results: Among 9468 new warfarin patients, 2746 (29%) were on PPIs, of whom 1424 (51.9%) had an indication for PPI prophylaxis by consensus recommendations. Among warfarin patients, PPIs were used by 59.3% of patients with a prior GIB, 48.1% of patients on glucocorticoids, 32.4% of patients on thienopyridines, and 31.0% of patients on aspirin. Overall, PPI use increased from 23.1% in 2009 to 31.7% in 2014 ($P < 0.001$). Recommendation-adherent PPI use increased from 24.2% in 2009 to 34.8% in 2014 ($P = 0.001$). Patients on PPIs were older (66.0 ± 14.2 vs. 63.5 ± 16.0), more often female (52.7% vs. 46.4%), and had higher HAS-BLED scores (3.0 ± 1.3 vs. 2.6 ± 1.3) and lower hemoglobin levels (11.5 ± 2.1 vs. 12.1 ± 2.2 g dL⁻¹), all $P < 0.001$. In adjusted analyses, prior GIB (OR 3.55, $P < 0.001$), glucocorticoid use (OR 2.41, $P < 0.001$), female gender (1.32, $P < 0.001$), aspirin use (OR 1.21, $P < 0.001$), and year of warfarin initiation (OR 1.06, $P < 0.001$) predicted PPI use.

Conclusion: In this large, multicenter study of patients referred to anticoagulation clinics, most patients at risk for GIB were not taking PPIs. Anticoagulation clinics may present an important opportunity for improving GIB prevention efforts.

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PO624-TUE

What bleedings predict HAS-BLED and HEMORR2HAGES scales in patients on long-term warfarin therapy?

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Background: Application of warfarin (W) is complicated because of bleedings (Bl) especially at the beginning of the treatment. HAS-BLED and HEMORR2HAGES scales are recommended for an assessment of bleeding risk.

Aims: To estimate whether HAS-BLED and HEMORR2HAGES scales predict single and recurrent Bl during long-term W therapy.

Methods: Our retro-prospective study involved 119 pts (72 males), aged 60.9 ± 9.6 years with atrial fibrillation or venous thromboembolism. Pts were followed at coagulation clinic. INR was measured monthly (TTR > 60%). Follow up period was 5.6 ± 3.4 years. All Bl were categorized as (i) single Bl with INR > 4.0 during first month of therapy; (ii) single Bl any time after first month of treatment and (iii) recurrent Bl. All pts were matched for HAS-BLED, HEMORR2HAGES scales. Analysis of CYP2C9 and VKORC1 (G3673A) genotypic variations was performed with PCR in all pts.

Results: In our investigation 39.5% pts ($n = 47$) had haemorrhagic complications, where 8 pts (17%) had single Bl with INR > 4.0 during first month of therapy, 12 pts (25.5%) had single Bl any time after first month of treatment and 27 pts (57.5%) had recurrent Bl. Pts with high risk of HAS-BLED and HEMORR2HAGES scales have significantly more often recurrent Bl ($P = 0.0048$; $P = 0.018$ accordingly), than pts with low risk of scales on the above. Correlation between all Bl complications, a single Bl with INR > 4.0 during first month of therapy, single Bl any time after first month of treatment and total score of HAS-BLED, HEMORR2HAGES scales was not found. Carriers of the allelic variants of VKORC1 and CYP2C9 (T/T or 2*/2*, 2*/3*, 3*/3* or combination of them) significantly more often have single Bl with INR > 4.0 during first month of therapy ($P = 0.011$).

Conclusion: In our study HAS-BLED and HEMORR2HAGES scales performed best in predicting recurrent Bl in pts on long term W therapy. Single Bl with INR > 4.0 during the 1st month of W therapy is associated with reduced warfarin metabolism (AA VKORC1 or/and allelic variants 2*/2*, 2*/3*, 3*/3* CYP2C9).

Disclosure of Interest: None declared.

PO625-TUE

Vitamin K antagonists favorably modulate fibrin clot properties in patients with atrial fibrillation as early as after 3 days of treatment: relation to coagulation factors and thrombin generation

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Background: Atrial fibrillation (AF) increases the risk of thromboembolism that is reduced by vitamin K antagonists (VKAs).

Aims: We sought to investigate changes in plasma fibrin clot phenotype at the onset of oral anticoagulation.

Methods: We studied 40 consecutive AF patients (aged 45–83 years, CHA₂DS₂-VASc score 3.0 ± 1.5) who started therapy with warfarin or acenocoumarol. Tissue Factor (TF)-based plasma fibrin clot permeability (K_s), clot lysis time (CLT), along with clotting factors (F), thrombin generation (TG) profiles, protein C (PC), and fibrin clot

morphology detected by a confocal microscopy were determined on days 3, 5, 7, 28, and 56 ± 1 since the first dose.

Results: AF patients had 16% higher K_s and 15% shorter CLT as early as on day 3 of VKA therapy compared with the baseline (both $P < 0.001$), reaching the plateau values on day 7 and 5, respectively. Confocal microscopy confirmed VKA-induced alterations to plasma fibrin clots. K_s , but not CLT was associated with TG parameters from the 5th day of the therapy. There were no associations between CHA_2DS_2 -VASc score and K_s or CLT at any time point. On days 28 and 56 there were inverse correlations of K_s with VK-dependent factors, while CLT correlated with FII, FVII, and FX only on day 56. Interestingly, FIX levels were the strongest determinants of K_s . Higher K_s values on days 1 and 3 were associated with further stable anticoagulation.

Conclusion: In conclusion, plasma clot density and lysis in AF patients are favorably modified as early as after 3 days of VKA administration, which might contribute to the antithrombotic effectiveness.

Disclosure of Interest: None declared.

PO626-TUE

Prevalence of anemia in patients under treatment with vitamin K antagonists

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Background: The use of vitamin K antagonist (VKA) is associated with bleeding events. Anemia often seen in these patients can be due to minor occult bleedings.

Aims: To evaluate the prevalence of anemia in patients under VKA.

Methods: Blood count and iron kinetics were measured. International normalized ratio (INR) and time on therapeutic range (TTR) were assessed. Anemia was defined as hemoglobin (Hg) value ≤ 13 g dL⁻¹ in men and ≤ 12 g dL⁻¹ in women. Mann–Whitney tests were used to compare continuous variables and Pearson χ^2 test for categorical variables.

Results: Two hundred and fifty-two patients were included in the study, 120 (47.6%) men and 132 (52.4%) women, with a median age of 72 years-old (range 62–79). All were in anticoagulation therapy for at least one year. 45.6% of them were anticoagulated due to atrial fibrillation. Overall prevalence of anemia was 23.4% ($n = 59$), 24.2% ($n = 29$) in men and 22.7% ($n = 30$) in women. No statistically significant differences were seen between anemic and non-anemic patients regarding gender. Ferritin ($P = 0.001$) and transferrin saturation ($P = 0.001$) were significantly lower in anemic men although between normal range, suggesting other causes rather than iron deficit. Regarding age, anemic patients were elder men ($P = 0.008$), in contrast with women in whom no statistically significant difference was observed. In general, TTR was not inferior in anemic vs. non-anemic patients, neither in men nor in women.

Conclusion: Overall prevalence of anemia was in accordance with a recent national population study (20.3%). Age seemed to not influence the prevalence of anemia on our population studied except for older men. Lower TTR is not a determinant of development of anemia. Anemic patients under VKA present lower normal iron kinetics thresholds so other causes of anemia must be excluded.

Disclosure of Interest: None declared.

von Willebrand Disease II

PO627-TUE

Genetic variations in exon 28 of von Willebrand's factor gene in Nigerian population

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Background: Although dysfunctions of von Willebrand's factor (VWF) are linked with many haemostatic disorders, little has been done to investigate these disorders in Nigeria.

Aims: Our objective was to molecularly characterize the exon 28 of the VWF gene in the three major ethnic groups of Nigeria.

Methods: We recruited 90 subjects, with 30 subjects from each of the ethnic groups, 45 had a history of bleeding. Questions included those used in the ZPMCB –VWD and the bleeding scores were calculated using the MCMDM-1 VWD scoring system. Platelet count, platelet indices, coagulation profile; Prothrombin time (PT), activated partial thromboplastin time (APTT), and FVIII: C activity were carried out. Also, VWF: Ag level and VWF: CB activity was done. Data was analysed using Graph pad Prism software version 5.03. 30 samples were sequenced. The Big Dye terminator chemistry was used to determine the nucleotide sequences of VWF gene (exon 28), at ACGT. Inc., USA.

Results: The mean platelet count in control and index cases were 224.7 ± 64.39 , 222.4 ± 61.88 . Also VWF: Ag, and VWF: CB activity differed significantly. Eight SNPs were identified, the SNPs rs216311, rs1800383 and rs1800386 associated significantly with bleeding in study subjects, with P value < 0.05 . rs1800386 occurred in all with bleeding history, no ethnic variations were noted.

Table 1 Sequence Variations Identified in exon 28 of VWF gene in the three major ethnic groups of Nigeria

SNP ID	Ref. Allele	Allele Change	Protein position	Residue change
rs216310	T	ACT→ACC	1547	T [Thr]=T [Thr]
rs1800385	G	GTG→TTG	1565	V [Val]=L [Leu]
rs1800384	A	GCA→GCC	1555	A [Ala]=A [Ala]
rs1800383	G	GAC→CAC	1472	D [Asp]=His [His]
rs1800386	A	TAC→TGC	1584	Y [Tyr]=C [Cys]
rs216311	A	ACC→GCC	1381	T [Tyr]=A [Ala]
rs216312				
rs1800381	G	CCG→CCA	1337	P [Pro]=P [Pro]

Conclusion: This is the first population based study for VWD in Nigeria. The three SNPs that associated significantly with bleeding are not pathogenic for VWD for our population. Studies are ongoing for full sequencing VWF gene in Nigerian population

Disclosure of Interest: E. Ezigbo Grant/Research Support from: The project described was partly supported by Award Number 1R24W008878 from the Fogarty International Centre, E. Ukaejiofo: None declared, T. Nwagha: None declared.

PO628-TUE

Clinical features and types of von Willebrand disease in women with menorrhagia referred to hematology clinic of Kermanshah

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Background: Menorrhagia is the most common symptom that is experienced by women with bleeding disorders. von Willebrand disease

(VWD) is the most common congenital human bleeding disorder that is manifested as a quantitative deficiency in (VWF) or dysfunction of this factor. VWD is more readily detected in women due to the presence of severe bleeding associated with menstrual cycles and childbirth.

Aims: The present study was carried out to find the frequency of VWD, its types, and clinical features of the disease among women with menorrhagia

Methods: The study comprised 482 women with menorrhagia. After excluding patients with confounding factors, 56 (11.6%) patients were evaluated for inherited bleeding disorders. For coagulation assays, venous blood samples were collected in tubes containing 0.109 M (3.2%) trisodium citrate in a ratio of 9 parts blood to 1 part anticoagulant and then centrifuged without delay at 1200 G for 15 min.

Results: Type 3 was the most frequent with a prevalence of 45.2% (14 out of 31), type 2 was the second prevalent type, 10 out of 31 (32.3%), and finally there were 7 out of 31 women (22.5%) with type 1. The assessment of blood loss during menstruation was done by checking detailed history of pad/tampon changing. In 15/31 (48.38%) cases, heavy blood loss, amounting to approximately more than 100 mL cycle⁻¹ was seen, whereas in the remaining 16/31 cases (51.61%), there was moderate loss of blood. In type 3, presentation was similar to patients with hemophilia having hemarthrosis (37.5%) and hematomas (43.7%).

Conclusion: We detected 31 (55.3%) patients with VWD. Type 3 of VWD was the most frequent subtype (45.2%) followed in frequency by type 2 (32.3%), and type 1 (22.5%). our study indicated that menorrhagia can be the first symptom of VWD. Therefore, rare coagulation disorders should be considered in women with idiopathic menorrhagia, particularly in regions with high rates of consanguinity.

Disclosure of Interest: None declared.

PO629-TUE

A phase III open-label, multi-center study with a plasma-derived von Willebrand factor/factor VIII concentrate to assess the pharmacokinetics, efficacy, and safety in pediatric subjects with von Willebrand disease (SWIFTLY-VWD study)

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Background: Voncento[®] comes with a low-volume and the solution contains a large proportion of high-molecular-weight VWF multimers with a von Willebrand Factor (VWF): factor VIII (FVIII) ratio of 2.4:1. The SWIFT ('Studies with von Willebrand factor/Factor VIII') program is evaluating this product in hemophilia A and VWD patients in accordance with the European clinical and pediatric guidelines.

Aims: This study evaluated the Pharmacokinetics (PK), efficacy and safety of a VWF/FVIII concentrate (Voncento[®], CSL Behring) in 17 pediatric subjects < 12 years with severe VWD who require treatment of non-surgical bleeding (NSB) or who received it for prophylaxis.

Methods: PK included a single intravenous dose of 80 IU kg⁻¹ body weight VWF:Ristocetin Cofactor (RCo) on Day 1 and 180. Thirteen subjects received Voncento[®] as on-demand therapy ($n = 6$: < 6 years; $n = 7$: 6–12 years old; $n = 6$: type 2A; $n = 7$: type 3 VWD) and 4 sub-

jects as prophylaxis therapy ($n = 3$: < 6 years of age; $n = 1$: 8 years old; $n = 3$: type 3 VWD; type 2A VWD once) for 12 months.

Results: PK parameters for VWF:RCo, VWF:Antigen, and VWF:Collagen Binding were similar between age groups (< 6 vs. 6–12 years) in the initial and repeat analyses. Excellent or good hemostatic efficacy was reported by the investigator for all 80 NSB events in the on-demand arm. In the prophylaxis arm NSBs were reported with 100% excellent or good efficacy treatment results. There were no relevant differences in the efficacy between subgroups by age or by VWD type. Voncento[®] was well tolerated. The adverse events seen were mild-moderate and consistent with the safety profile for this product in adults. There were no cases of anaphylactic reactions or angioedema, development of FVIII/VWF inhibitors, thromboembolic events, or viral infectious disorders.

Conclusion: This contemporary comprehensive development program evaluating Voncento[®] across all ages demonstrates safety and efficacy for treatment and prevention of bleeding in pediatric subjects with severe VWD.

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PO630-TUE

von Willebrand disease and menorrhagia: multiple treatment options for different patients

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Background: Menorrhagia is defined as heavy and prolonged bleeding during the menstrual period without abnormal periodicity and a common problem in von Willebrand Disease (VWD). Management of this condition has traditionally been responsibility of the hematologists. Women with VWD frequently have menorrhagia and report lower quality of life and absenteeism from school or work during menstruation.

Aims: In this report we evaluated menorrhagia issue among all patients with vWD and related therapeutic options.

Methods: Ege University Children's Hospital is the biggest center in Turkey for bleeding disorders. We have 152 patients with VWD registered to our center (87 Female, 65 Male; mean age: 16 yr; range: 1.5–28). 111 of these patients are type-1 (73%), 25 type-2 (17%) and 16 type-3 (10%). Among 86 female VWD patients, 65 are type-1 (74%), 12 are type-2 (14%) and 10 are type-3 (12%).

Results: Among 87 female patients with vWD, 60 have menstrual cycles (70%). 34 of them have menorrhagia (57%). 23 are type-1 (67%), 6 are type-2 (17%) and 5 are type-3 (15%). Frequency of menorrhagia was 50% in type-1, 67% in type-2 and 100% in type-3. Most frequent treatment options were tranexamic acid (TA), desmopressin (DDAVP) and VWF-containing factor VIII concentrates (vWFs) and oral contraceptives (OC). Treatment modalities according to VWD types; TA used in 9 Type-1 patients (with OC in 4 pts), DDAVP plus TA used in 12 Type-1 pts (with OC in 2 pts), and 4 Type-2 pts. vWFs plus TA used 2 pts with Type-1 (with OC in 1 pt), 2 pts with Type-2 and 5 pts with Type-3. OCs are used for 3 patients with vWD.

Conclusion: In conclusion, women with VWD frequently have menorrhagia problem and need for treatment. Severe or mild bleeding episodes can be controlled by different agents. Successful management of VWD with menorrhagia requires the combined expertise of gynecologist and hematologist, most likely in specialized centers such as hemophilia treatment centers or expert university hospitals.

Disclosure of Interest: None declared.

PO631-TUE

Unexpected finding of low von willebrand factor levels or von Willebrand disease in pediatric patients with scoliosis

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Background: Patients with scoliosis have more blood loss during spinal instrumentation than other pediatric surgical populations. We began including platelet function analysis using PFA-100™ (PFA) to the routine preoperative coagulation testing (i.e. prothrombin time, activated partial thromboplastin time, and fibrinogen assays) in hopes of optimizing pre- and intraoperative hemostasis, and thereby reducing blood loss.

Aims: Demonstrate that, regardless of bleeding history, PFA has utility as a screening tool in pediatric scoliosis patients.

Methods: Abnormal PFA results were defined as closure times > 183 s for collagen/epinephrine and/or > 126 s for collagen/ADP; upper limits were established from the mean $\pm$ 2 standard deviations of normal donor samples. Abnormal PFA results led to a reflex von Willebrand factor (vWF) panel. Low vWF and von Willebrand disease (vWD) were defined as ristocetin cofactor activity 30–50% and < 30%, respectively.

Results: Since initiation of PFA as part of the preoperative workup, we screened 20 total patients and identified 4 cases (3 female, 1 male, age range 6–12 years old) with abnormal PFA results. None of these 4 patients had a personal or family history of bleeding. Their scoliosis diagnoses included neuromuscular ($n = 2$), secondary to trauma ($n = 1$), and genetic etiology ($n = 1$). Two patients received anticonvulsants not reported to be associated with acquired vWD. In two cases, PFA closure time was prolonged in both agonists; the other two cases showed prolongation in only one channel. By reflex testing, three were consistent with low vWF and one with vWD; multimer analysis was normal in all.

Conclusion: PFA screening followed by work up for vWD prior to scoliosis surgery identified 4 patients with low vWF/vWD, which was unexpectedly high prevalence. Further studies are needed to determine if there is association between low vWF/vWD and scoliosis and to establish the utility of PFA in this patient population.

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PO632-TUE

Percentage of platelet aggregates and macroplatelets: a potential useful laboratory parameter for predicting bleeding in von Willebrand disease type 2B (VWD2B) with the P.VA1361MET mutation

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Background: VWD Type 2B is an autosomal dominant bleeding disorder caused by a gain-on-function mutation in the A1 domain of von Willebrand factor (VWF). The bleeding phenotype observed in these patients may be due to the combination of a low platelet count and the presence of abnormal VWF.

Aims: We present here a large Argentinean family with VWD2B caused by the p.Val1361Met mutation and macrothrombocytopenia and propose a relationship between the percentage of macroplatelet and platelet aggregates with bleeding score.

Methods: The percentage of macroplatelets was calculated on May-Grünwald-Giemsa stained peripheral blood films in three different blood samples from each patient by the same expert operator. Platelet aggregates were quantified in the same way and arbitrarily separated into three groups a) small aggregates containing 2–3 platelets, b) medium aggregated containing 4–6 platelets and c) large aggregates containing > 6 platelets.

Results: The percentage of macroplatelets in the index case and his daughter were 35 and 37% when compared to other affected members (his father and two brothers) that showed 19, 25 and 30% respectively. Moreover, these two patients also showed a higher percentage of large platelet aggregates when compared to the rest of the affected family members. Interestingly, in a high percentage (> 75%) of platelet aggregates, small, medium or large, no macroplatelets were observed indicating a preference for normal size platelets to form aggregates. When compared to the bleeding scores (ISTH-BAT), the patients with the higher number of macroplatelets and aggregates exhibited higher bleeding scores suggesting a direct correlation.

Conclusion: Our results in this family with VWD Type 2B due to p.Val1361Met suggest that the percentage of macroplatelet and platelet aggregates are associated with increased bleeding tendency. Larger studies in other families will be necessary to confirm these preliminary findings.

Disclosure of Interest: None declared.

PO633-TUE

Development of aplastic anemia in a patient with acquired von Willebrand's syndrome and autoimmune preponderance

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Background: This 77 year old lady was diagnosed to have inherited mild von Willebrand's Disease (VWD) type I at the age of 53 years (heterozygous for an A>G substitution at nucleotide c.4138 of the von Willebrand's Factor gene). Clinically, she had a life-long history of bruising and easy bleeding. There was also a strong family history of myasthenia gravis (MG). Eighteen years later, she had worsening spontaneous bruising. VWF parameters were much lower than baseline values and her VWF:RCo inhibitor assay was positive. She was diagnosed additionally to have Acquired von Willebrand's Syndrome (AVWS) as well as Monoclonal Gammopathy of Undetermined Significance (MGUS). Her AVWS was managed with good response to monthly intravenous immunoglobulin and her paraprotein remained stable. In Sept 2014 she began developing mild pancytopenia associated with worsening bruising. Over the next few weeks this evolved into frank pancytopenia with haemoglobin of 82 g L⁻¹, WBC 1.4×10^9 L⁻¹, neutrophils 0.4×10^9 L⁻¹, and platelets of 8×10^9 L⁻¹.

Aims: This report highlights an autoimmune predisposition in this patient whose management of AVWS was complicated by multiple autoimmune conditions.

Methods: Standard analytical methods for the conditions described were performed.

Results: She had persistent lupus anticoagulant and anticardiolipin antibodies throughout. Furthermore, she had persistent acetylcholine receptor antibodies without signs of MG. There was laboratory evidence of both inherited and acquired VWS. Her paraprotein was

8 g L⁻¹ (IgG kappa). Her bone marrow was markedly acellular with plasma cell neoplasia (10% of disease burden). She was also found to have a PNH clone by peripheral blood flow cytometry.

Conclusion: As reported before, this patient is likely to be the first patient who developed AVWS on the background of MGUS and inherited VWD. To our knowledge, this is the first report of aplastic anemia development in a patient with AVWS and a strong autoimmune preponderance.

Disclosure of Interest: None declared.

PO634-TUE

Relationship between bleeding tendency and the affected domain with mutations responsible of VWD2M phenotype

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Background: Type 2M von Willebrand disease (VWD2M) is characterized by VWF:RCo/VWF:Ag < 0.6, and normal multimers. Mutations (Mt) are mainly in the A1 domain (A1), and, in a minor extent, in the A2. Variable bleeding tendency is common in patients (P)

Aims: To correlate bleeding tendency with Mt location within affected members (AFM) in families (F), and determine the Mt penetrance and VWD prevalence (Pr) within F with ≥ 2 generations (G).

Methods: Bleeding score (BS), major bleeding (MB), bleeding time (BT) (min), factor VIII (FVIII) and VWF (IU dL⁻¹), propeptide/VWF:Ag (VWFpp ratio). We selected 9F; n = 66; AFM=44 (66.6%)

Results: A1 Mt: F = 5 (n = 28); AFM=20. Diagnosis age: females (fem): median (md)=17 yr; males: md=11 yr. P with MB=30%. P with abnormal BS=66.6%; fem=72.7%; males=57%. p.F1293C: 1F; n = 2; 1G; AFM=100%; p.R1374C: 3F; n = 20; 2 and 3G; AFM=70% p.A1437T: 1F; n = 6; 3G; AFM=66.6%. BT=9.2 ± 4; FVIII=51 ± 23; VWF:Ag=26 ± 11; VWF:RCo=7.2 ± 4.5; VWFpp=2.7 ± 0.6. Pr=69.2%, with no differences between Mt.

A2 Mt: F = 4 (n = 38), AFM=24. Diagnosis age: fem: md=20 yr; males: md=12 yr. P with MB=41.6%. P with abnormal BS=66.6%; fem=82%; males=54%. p.L1503P: 1F; n = 4; 3G; AFM=50%; p.E1549K: 1F; n = 28; 4G; AFM=64.3%; p.R1564W: 1F; n = 2; 1G; AFM=50%; p.I1628T: 1F; n = 4; 2G; AFM=75%. BT=8.2 ± 3.3; FVIII=55 ± 22; VWF:Ag=59 ± 21; VWF:RCo=7.9 ± 6.5; VWFpp=1.5 ± 0.7. Pr=63.8%, with no differences within Mt

Conclusion: P from A1 group Mt showed low VWF:Ag and high VWFpp ratio (p = 0.000 respectively) suggesting shortened VWF survival; so, it is important to make the VWFpp in P, in order to know if use of low purity blood concentrates is required. Despite the shorter VWF survival, these P did not show more severe clinical symptoms than those from A2 group. In A2 group there were more P with MB, but not significant (P=ns; RR=1.2). BT, FVIII, and VWF:RCo did not show differences between A1 and A2 group P. Both A1 and A2 Mt were present in all G, showing complete penetrance, making mandatory the family study. Prevalence of VWD2M in A1 group is higher, but not significant.

Disclosure of Interest: None declared.

PO635-TUE

Characterization of von Willebrand disease subtypes in 303 patients – a single centre study over the past 10 years

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Background: Diagnosis of vWD can be complicated by the fact that vWF is an acute phase reaction protein and hence accurate classification of VWD requires the appropriate and critical evaluation with correlation of several laboratory tests.

Aims: To characterize the vWD subtypes diagnosed at the Sultan Qaboos University Hospital (Muscat, Oman).

Methods: Patients referred to our center for VWD work up from 2004 through 2014 were included in this study and have been characterized using the current ISTH subcommittee guidelines (JTH, 2006; 4: 2103). Laboratory diagnosis of VWD was made by FVIII:C assay [chromogenic method], VWF antigen (VWF:Ag), VWF ristocetin cofactor, VWF Collagen binding activity, VWF factor VIII binding activity, ristocetin induced platelet agglutination and multimer analysis. A cut off of < 0.6 in the VWF: RCo/Ag ratio and VIII/vWF:Ag ratio was used to differentiate type 1 VWD from type 2 and to characterize type 2N respectively.

Results: A total of 303 VWD patients (n, [%]) were included in this analysis showing the following distribution: type 1: 203 (67.0%); type 2A: 35 (11.5%); type 2B: 3 (0.9%); type 2M: 12 (4.2%); type 2N: 27 (8.8%); type 3: 23 (7.6%), total 303 (100%). In the Type II vWD patients, there was variable clinical bleeding phenotype that was not correlated with the measured factor levels. However, there was a good correlation with multimer analysis and the vWF type 2 subtype. The mean levels of F:VIII:C, vWF:Ag, vWF:RiCoF, vWF:CBA in the 2A, were 0.57, 0.42, 0.24, 0.21 u mL⁻¹ respectively, in the 2 M they were 0.72, 0.61, 0.18, 0.43 μ mL⁻¹ respectively, in the 2N they were 0.32, 0.79, 0.71, 0.78 u mL⁻¹ respectively.

Conclusion: vWF factor levels were poor predictors of the clinical bleeding phenotype in Type II vWD patients, in contrast to type III vWD patients. However, in Type I vWD patients, low levels of vWF factor levels correlated well with the incident bleeding diathesis. Overall, there was a better correlation with multimers /triplet structure and the vWD subtypes.

Disclosure of Interest: None declared.

PO636-TUE

VWD2B phenotype: its prevalence within families and bleeding tendency in affected members

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Background: von Willebrand disease type 2B (VWD2B) is characterized by gain-of function mutations (Mt), low platelet count (PC), ristocetin induced platelet aggregation at low concentration (RIPA).

Aims: To correlate bleeding tendency in affected members (AFM) in families (F), and also determine Mt penetrance and VWD prevalence (Pr) in F with ≥ 2 generations (G).

Methods: We have 12F, with 36M (AFM=28). Bleeding score (BS), major bleeding (MB), PC (×10⁹ L⁻¹), bleeding time (BT) (min), VWF

(IU dL⁻¹), lower RIPA concentration (mg mL⁻¹), VWFpp/VWF:Ag (VWFpp).

Results: Following Mt were described: p.R1304V: atypical VWD2B = 1F; *n* = 3; 2G; AFM=3; AFM with: abnormal BS=66.6%, both normal PC and multimers=100%, RIPA 0.4 = 33.4%, 0.5 = 66.6%, MB = 33.3%. BT=7.7 ± 2; VWF:Ag = 24 ± 6.7; VWF:RCo = 27 ± 12; RCo/Ag = 1.1 ± 0.2; VWFpp = 2.5 ± 0.3 (normal value=1.5 ± 0.6); PC=192 ± 26.Pr=100%. p.R1306W = 2F; *n* = 6; 3G; AFM = 5; AFM with abnormal BS = 100%, low PC and absence HMWM = 100%, RIPA 0.5 = 66.6%, 0.7 = 33.3%, MB = 60%. BT = 7.2 ± 1; VWF:Ag=53 ± 14; VWF:RCo=43 ± 16; RCo/Ag=0.8 ± 0.2; VWFpp=2.2 ± 1.5; PC=90 ± 41.Pr=83.3%. p.R1308C = 4F; *n* = 8; 2G; AFM = 7; AFM with abnormal BS = 28.6%, low PC = 29%, absent HMWM = 100%, RIPA 0.3 = 33.3%, 0.4 = 16.6%, 0.6 = 16.6%, 0.7 = 33.3%, MB = 28.6%. BT = 10 ± 2; VWF:Ag = 54 ± 21; VWF:RCo = 12 ± 5.4; RCo/Ag = 0.2 ± 0.1; VWFpp = 2 ± 0.6; PC = 161 ± 76.Pr = 100%. p.S1310F = 1F; *n* = 5; 2G; AFM = 3; AFM with abnormal BS = 100%, low PC and absent HMWM = 100%, RIPA 0.4 = 66.6%, 0.7 = 33.4%, MB = 66.6%. T = not done; VWF:Ag = 66 ± 19; VWF:RCo=52 ± 17; RCo/Ag=0.8 ± 0.1; VWFpp = 2.1 ± 0.2; PC = 66 ± 63. Pr=100%. Parents without Mt: probable *de novo* in all AFM. p.V1316M = 4F; *n* = 14; 3G; AFM = 10; AFM with abnormal BS = 100%, low PC = 70%, absent HMWM = 100%, RIPA 0.3 = 37.5%, 0.5 = 50%, 0.7 = 12.5%, MB = 60%. BT = 11 ± 3; VWF:Ag = 51 ± 20; VWF:RCo = 28 ± 12; RCo/Ag = 0.5 ± 0.2; VWFpp = 2.0 ± 0.7; PC = 119 ± 108.Pr=77.8%. Parents without Mt: probable *de novo* in the AFM of one F

Conclusion: A 45% of AFM showed VWFRCo/VWF:Ag > 0.6. MB was related to PC: *P* = 0.034; likelihood ratio of +test=2.7; RR=2.66. Presence of Mt in all G (complete penetrance) makes mandatory family study. Similar prevalence of VWD2B was seen in all F. Low PC showed to be a risk factor of MB in VWD2B

Disclosure of Interest: None declared.

PO637-TUE

von Willebrand disease (VWD), experience of Algeria in beni messous center in Algiers

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Background: In Algeria a big step was made for the coverage of the hemophilia disease many things remains to make for the other bleeding disorders. In recent years the disease Willebrand is better known and better taken care.

Aims: von Willebrand disease (VWD), experience of Algeria in Beni Messous center in Algiers.

Methods: One hundred and seventy-one patients are attending in Algiers Beni Messous center, 105 (61%): female, 66 (39%): Male. 60 (35%) patients with severe VWD type 3 (50% female, 50% male) characterized by a severe quantitative defect and very low levels of both vwf and fviii the symptoms are highly variable: nosebleeds, gumbleeds, menorrhagia, metrorrhagia.

Results: VWD required special gynaecological and obstetric management, oral contraceptives can help to control the menstruation periods, 2 (type 3) patients received prophylactic treatment before ovulation period the median dose administered was 40 IU kg⁻¹, 6 patients with type 3 presented ovarian cyst ruptures, 12 pregnant women (6 with VWD type 1, 6 with VWD type 3) required hospitalization, all patients type 3 required to cover (delivery or caesarean section), 2 patients presented bleeding in post partum were transfused with packed RBCs. 4 received prophylaxis, the dosage and duration of treatment were tailored to the type of procedures the dosages of VWF: RCo administered per infusion (30 -60 IU kg⁻¹). 21 (13 TYPE 3, 8

TYPE 1) patients were treated to prevent bleeding (3) surgical procedures were orthopedic (4) gynecological/obstetrical, (5) gastrointestinal, (4) skin surgery, (5) circumcision. Co-administration of fviii was given with VFW for the first infusion in all cases median infusion dosage VFW (50 IU kg⁻¹), VFW were repeated as necessary, a larger number of infusions required. 17 (type 3) patients received (short or long-term) prophylactic treatment before an exposure to situations associated with a high risk of bleeding the median dose administered was 50 IU kg⁻¹.

Conclusion: Follow up patients with vwd should be performed in specialised centres in Algeria many things remains to make.

Disclosure of Interest: None declared.

PO638-TUE

Two unusual cases of type 2N von Willebrand disease

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Background: von Willebrand's disease (VWD) is a heterogeneous disorder caused by either quantitative (types 1 and 3 VWD) or qualitative (type 2 VWD) abnormalities of von Willebrand factor (VWF). VWF has two main haemostatic roles: as an adhesive protein anchoring platelets to sub-endothelial components and as a carrier and stabiliser of factor VIII (FVIII) in the circulation. The functions of VWF have been located to specific domains. Regions essential for FVIII binding reside in two regions of the VWF protein: the D' (amino acids 769–865 in pre-VWF) and D3 (amino acids 866–1242 in pre-pro-VWF) domains, encoded by exons 18–27. Type 2N VWD refers to variants that have a decreased affinity for FVIII.

Aims: This report describes the molecular defects underlying 2 families with type 2N VWD.

Methods: DNA samples were sequenced on an ABI 3730 and analysed using Mutation Surveyor a variant analysis software and Alumut a variant interpretation software.

Results: *Family 1: Index case:* VWF:Ag 0.26 IU mL⁻¹, VWF:RCo 0.25 IU mL⁻¹, FVIII:C 0.11 IU mL⁻¹, VWF:FVIII:B < 20%. High molecular weight multimers were unusually absent. Analysis of the VWF gene sequence detected two novel mutations, in exon 19 (c.2546G>T, p.Cys849Phe) and intron 50 (c.8155 + 6T>A)

Family 2: Index case: VWF:Ag 0.39 IU mL⁻¹, VWF:RCo 0.39 IU mL⁻¹, VWF:CBA 0.30 IU mL⁻¹, FVIII:C 0.29, VWD: FVIII:B 18%. High molecular weight multimers were normal. Analysis of the VWF gene sequence detected 2 mutations, a known mutation in exon 24 (c.3159G>T, p.Glu1053His) and a novel mutation in exon 41 (c.6979T>A, Cys2327Ser)

Conclusion: Both families have atypical genetic mutations leading to type 2N VWD. Typically, mutations are located either within the N terminal D'/D3 assembly FVIII binding region of VWF (> 95% of cases) or at the furin cleavage site for removal of the VWF propeptide. This report highlights that full VWD gene screening may be required to explain the 2N VWD phenotype rather than only hotspot analysis.

Disclosure of Interest: None declared.

PO639-TUE

Health-related quality of life (HRQOL) and health-economic assessment in patients with von Willebrand disease in France: the WISH-QOL studyBorel-Derlon A¹, Goudemand J², Boyer-Neumann C³, Fressinaud E⁴, Ternisien C⁵, Trossaert M⁵, Veyradier A⁶, WiSH-QoL Study Group⁷, Martinez C⁸, Chatelanaz C⁸ and Von Mackensen S⁹¹Hemophilia Treatment Centre, Hospital Cote de Nacre, Caen;²Hematology Unit, Université de Lille, Lille; ³HematologyLaboratory, A. Beclere Hospital, Clamart; ⁴Inserm U1176,Kremlin Bicêtre; ⁵Hemophilia Treatment Centre, Nantes;⁶Hematology Unit, Lariboisière Hospital, Paris; ⁷French HTC;⁸LFB, Les Ulis, France; ⁹University Medical Centre, Hamburg-

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Background: VWD patients with bleeding phenotype, requiring treatment, have been estimated as about 100 cases *per million persons*. VWD and its treatment impact on patients' health-related quality of life (HRQoL) and may affect the everyday life of their families.

Aims: The WISHQoL study aims to assess HRQoL in VWD patients of any age and to evaluate associated costs related to VWD management.

Methods: The prospective cohort study will enrol at least 350 patients with inherited VWD type 1 (VWF:Ag < 30%), type 2 and type 3 who will be followed for 2 years.

Results: Clinical phenotype such as bleeding score (Tosetto score), biological profile and genotype will be documented. Patients are treated with a Von Willebrand factor devoid of Factor VIII. This WiSH-QoL study is conducted with the French Reference Centre for von Willebrand Disease (CRMW). Patients will fill in a booklet collecting information on socio-demographic and resources consumption. Clinical characteristics and therapeutic approach will be recorded. HRQoL is assessed with the generic SF-36 (for adults) and the chronic-generic DISABKIDS Short Form (for children and adolescents) and the VWD-specific VWD-QoL (for adults, children and adolescents). In addition treatment satisfaction is assessed. A proxy version of these VWD-specific questionnaires (for parents) and the Impact on Family (IOF) Scale are dedicated to caregivers of children with VWD. Special attention will be given to specific clinical subgroups such as women during the reproductive period etc. Thirty centres have accepted to participate. After the first 4 months of enrolment, 17 patients have been included.

Conclusion: With the results of the WiSH-QoL study, especially the VWD-specific evaluation of HRQoL and treatment satisfaction a deeper insight into the patients' real daily life, their perception of well-being and their specific health care needs will be gathered. This study will provide an important contribution to HRQoL knowledge in VWD patients and their families.

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PO640-TUE

POor response to 1-desamino-8-D arginine vasopressin (DDAVP) test in a patient with von Willebrand disease type 2M (VWD2M) with P.GLU1549LYS mutationGuglielmone H^{1,2}, Frontröh J³, Minoldo S¹, Hepner M³, Sabagh M⁴ and Jarchum G⁵¹Laboratorio de Hematología, Sanatorio Allende; ²Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas,Universidad Nacional de Córdoba, Córdoba; ³Laboratorio de

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Background: Type 2 VWD is subdivided into categories (2A, 2B, 2N and 2M) based on structural and functional abnormalities. Type 2M VWD has been reported to show a variable response to DDAVP depending on the mutation. In our country there is no information on the response to DDAVP in patients with VWD2M.

Aims: To describe the phenotypical data of response to DDAVP test in a patient with VWD2M which carries the mutation p.Glu1549Lys in von Willebrand factor (VWF).

Methods: Patient was a 21 yo male who had prolonged bleeding after tooth extractions and a lifelong history of easy bruising and epistaxis. History was positive in his father and elder sister. Hematological examination revealed a normal platelet count and prothrombin time but a prolonged aPTT (47 s; normal range: 26–37 s). Low VWF:RCo activity but normal VWF:Ag were observed (0.30 and 0.53 IU mL⁻¹ respectively; normal values: 0.50–1.50 IU mL⁻¹). He also showed a decreased platelet aggregation induced with ristocetin (1.25 mg mL⁻¹). DDAVP test (0.3 µg kg⁻¹ i.v.) was performed. Platelet count and levels of factor VIII, VWF:RCo and VWF:Ag were determined before and 60 min after infusion. Direct sequence of exon 28 was carried out using genomic DNA.

Results: Before and 60 min after drug administration, platelet count was: 173 and 162 × 10⁹ L⁻¹, factor VIII: 0.26 and 0.58 IU mL⁻¹, VWF:Rco: 0.20 and 0.23 IU mL⁻¹ and VWF:Ag: 0.48 and 0.68 IU mL⁻¹, respectively. A heterozygote c.4545G>A change resulting in a p.Glu1549Lys mutation was found.

Conclusion: We observed a poor of response to DDAVP in our patient with vWD2M and p.Glu1549Lys mutation. A correlation of response to DDAVP with other mutations can only be speculated and further studies are needed to resolve this issue.

Disclosure of Interest: None declared.

PO641-TUE

Allele-specific silencing of the von Willebrand factor gene to ameliorate the von Willebrand disease phenotype

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Background: von Willebrand disease (VWD) is the most common inherited bleeding disorder caused by qualitative or quantitative defects of von Willebrand factor (VWF). Patients suffer from mucocutaneous, post-traumatic or surgical bleedings. The more severe forms may be associated with joint bleeds, thrombocytopenia or vascular malformations like gastrointestinal angiodysplasia. Treatment of VWD is focussed on increasing VWF plasma levels which, however,

does not act upon the associated phenotypes of thrombocytopenia and angiodysplasia.

Aims: We aim to develop a therapy for VWD that will increase the levels of normal VWF as well as ameliorate the associated phenotypes. As most VWD is caused by dominant-negative *VWF* mutations, we hypothesize that silencing the mutated allele will have a positive effect on VWF characteristics.

Methods: siRNAs will be used to prove the principle of allele-specific silencing. The siRNAs can be designed to target the specific *VWF* mutation, but a more optimal approach would be to target frequently found heterozygous single nucleotide polymorphisms (SNPs). The siRNAs will be tested on HA-tagged VWF constructs transfected in HEK293 cells and on patient-derived blood outgrowth endothelial cells (BOECs). Since BOECs are patient-specific and harbour the mutation in its native environment, they are the cells of choice to confirm amelioration of the phenotype. This will be examined by measuring VWF levels, VWF multimers, VWF string formation and immunofluorescence staining of the cells.

Results: Tagged VWF constructs have been made. Wild type HA-tagged VWF produce normal multimers and pseudo-Weibel-Palade bodies in HEK293 cells. Three allele-specific siRNAs targeting one SNP have been individually tested on VWF transfected HEK293 cells. Up to 65% allele-specific suppression of *VWF* was observed.

Conclusion: These first results show that it is feasible to induce allele-specific silencing of *VWF* by siRNAs. The efficiency and specificity will be further optimized and more siRNAs will be tested.

Disclosure of Interest: None declared.

PO642-TUE

Evaluation of a new automated ristocetin cofactor assay on the STA-R evolution analyzer

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Background: Ristocetin cofactor activity of von Willebrand Factor (vWF:RCo) is used as a screening to estimate vWF function and to detect von Willebrand Disease (vWD). Since vWF:RCo is considered as the reference method to assess vWF activity, there is a need for an automated assay on the routine coagulation analyzer.

Aims: Evaluation of a new automated vWF Ricof assay on the STA-R Evolution compared with a reference method.

Methods: A CLSI EP-5 validation protocol was performed with the new ABP vWF Ricof assay (Stago) on a STA-R Evolution (Stago) and furthermore a method comparison was performed with the reference vWF:RCo (Siemens) on a BCS XP (Siemens). The principle of the ABP vWF Ricof assay is to measure the ability of patient vWF to agglutinate formalin-fixed platelets in the presence of ristocetin. Subsequently agglutination is measured on a STA-R evolution analyzer and vWF:RCo activity is obtained after interpolation against a calibration curve obtained from reference plasmas.

Results: Among 180 tests were performed in duplicate, the average difference (absolute value) between duplicate was < 2% suggesting that single testing can be used safely. Between-run reproducibility (EP-5) showed CVs between 5% and 13%. Highest CVs were obtained for vWF:RCo activity results above 80%. Regarding batch-to-batch reproducibility, 2 lots were tested and displayed a high level of correlation ($y = 0.9978 - 1.33$, $r^2 = 0.9615$). Both 2 lots of ABP vWF Ricof assay showed a good level of correlation to another commercially available vWF:RCo assay ($y = 1.09x + 4.7$; $r^2 = 0.914$ and $y = 1.02x + 10.6$; $r^2 = 0.893$ respectively).

Conclusion: The new vWF Ricof assay automated on STA-R Evolution displays satisfactory analytical performances and ergonomics. It is well correlated to a reference method and takes benefit from accept-

able lot-to-lot consistency. These characteristics suggest it could be used as a first line assay to detect vWD in hemostasis laboratories on routine STA-R Evolution analyzers.

Disclosure of Interest: None declared.

PO643-TUE

How well can laboratories diagnose type 2N VWD?

Data from a UK NEQAS (blood coagulation) exercise

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Background: Type 2N von Willebrand disease is a rare subtype of VWD in which a mutation (mostly in exons 18–20) of the *VWF* gene leads to impaired binding of the VWF molecule to FVIII, and consequent shortened half-life of FVIII. Routine laboratory assays will show reduced FVIII levels, but normal VWF, mimicking a diagnosis of haemophilia A. Differences in the clinical approach to management of patients with type 2N VWD and Haemophilia A, together with the implications for genetic counselling for this autosomal defect, reinforce the need to differentiate these disorders with reliable FVIII binding assays. However, there are few data about the accuracy or precision of FVIII binding assays for type 2N VWD.

Aims: The aim of this multicentre study was to evaluate the ability of laboratories to diagnose Type 2N VWD with FVIII binding assays.

Methods: Following informed consent, citrated plasma was obtained from a normal donor (sample 1) and a donor with 2N VWD (sample 2), buffered, lyophilised, and distributed to 15 UK NEQAS participants with a request to perform FVIII binding assays. Participants were asked to provide method details, and an interpretation of their results.

Results: Results were received from 12 centres (6 UK and 6 non-UK). 7 centres (5 using a Stago method, 2 using laboratory-developed methods) returned quantitative results, reported as %. For sample 1, results ranged from 90–126%, for sample 2 from 0–4%. 5 centres either reported results as a ratio or qualitative results. All centres correctly identified sample 1 as a normal sample, and sample 2 as a 2N VWD sample.

Conclusion: Despite the rarity of this disorder, the small number of centres performing FVIII binding assays (12 out of 1050 UK NEQAS participants), and the infrequency with which these laboratories perform this assay (from 3 to 40 per year), acceptable precision and 100% diagnostic accuracy was observed in this study.

Disclosure of Interest: None declared.

PO644-TUE

POstoperative bleeding and other outcomes in children with von Willebrand disease or low von Willebrand factor undergoing adenotonsillectomy

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Background: Children with von Willebrand disease (VWD) have increased risk for bleeding after adenotonsillectomy. Published guidelines for the management of these patients are vague.

Aims: To review short term outcomes in patients with VWD or low von Willebrand factor activity (low VWF) treated under a uniform institutional protocol.

Methods: We performed a retrospective chart review in children with VWD or low VWF treated via the Texas Children's Hospital institu-

tional protocol, which utilizes desmopressin (DDAVP) prior to and at hours 12, 24, 48, 72 after procedure and at days 7, 9 and 11, and aminocaproic acid for up to 14 days. Patients were kept for minimum of overnight observation.

Results: Between October 2010 to December 2014, 11 children with VWD or low VWF underwent an adenotonsillar procedure and were treated with this protocol. Patients had median age of 7 years (range 3 to 17 years) and baseline ristocetin cofactor activity of 32% (range 12 to 44%). Estimated blood loss during surgery was minimal. Patients received a median of 6 doses of DDAVP (range 4 to 8) and 14 days of aminocaproic acid (range 7 to 14 days). The median length of hospital stay after surgery was 3 days (range 1 to 4 days). Eight patients (73%) had hyponatremia within 48 h after surgery (median 132 mmol L⁻¹, range 128 to 134 mmol L⁻¹) leading to holding DDAVP doses, but none experienced neurologic dysfunction or seizures. Bleeding within the first 24 h after surgery did not occur in any patients. Three (27%) children experienced delayed minor bleeding which resolved without additional intervention.

Conclusion: No episodes of major bleeding or need for intervention occurred with use of a straightforward protocol using DDAVP and aminocaproic acid in a group of patients with VWD or low VWF at risk for postoperative bleeding from adenotonsillectomy. Although mild hyponatremia occurred in a majority, no patients were symptomatic and all instances were successfully managed with conservative measures.

Disclosure of Interest: None declared.

PO645-TUE

Willebrand disease molecular diagnosis by high throughput sequencing: what's about the highly homologous pseudogene ?

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Background: von Willebrand Disease (VWD) is a common autosomally inherited bleeding disorder which results from a quantitative or qualitative deficiency of von Willebrand factor (VWF). The VWF gene (*VWF*) consists of 52 exons which gives rise to a 9 kb mRNA. Systematic identification of the causal VWD sequence variations has been hampered by the large size of the *VWF*, but the main difficulty resides on a 97% homologous partial pseudogene (*VWFPI*) from exon 23 to exon 35 (21 kb of genomic DNA). Sequence variations were found all along the gene (60% were novel on the French cohort VWD). Then cost and time consuming of Sanger method sequencing remain a serious obstacle for most diagnostic laboratories. However, Next Generation Sequencing (NGS) have radically changed the technical perspectives

Aims: Then we have performed NGS to evaluate the accuracy of this resequencing technique and evaluated the difficulties the pseudogene may provide for VWD diagnosis.

Methods: We have selected the Agilent HaloPlex target enrichment system to prepare library samples running out on illumina *MiSeq* platform. The HaloPlex probes design comprised *VWF* exons, exons-introns boundaries, 5' and 3' UTRs and the known promoter.

Results: This design foresee a coverage rate of 95.84% of the coding gene. 100 patients have been analysed and results were compared to capillary sequencing on each exon for 11 patients and each NGS sequence variations and their homo/heterozygous status were confirmed for the other patients by Sanger sequencing

Conclusion: Results shown that Agilent technology not allow the pseudogene coverage for exons 26 and 28 (the largest and pseudogene

most homologous exons). Then, our current strategy included an exons 26 and 28 Sanger sequencing before performing HaloPlex technology.

Disclosure of Interest: None declared.

PO646-TUE

Clinical and laboratory detection of transient neutralizing antibodies in severe von Willebrand disease on long term prophylaxis

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Background: Patients with type 3 VWD and some severe type 2 and type 1 VWD may benefit from long term prophylaxis (LTP) when bleeding is frequent and severe. Neutralizing antibodies to VWF have been reported in 10–15% of VWD type 3 patients.

Aims: To describe two cases on LTP that developed a transient neutralizing antibody associated to bleeding symptoms

Methods: FVIII:C, VWF:Ag, VWF:RCO, and mixing studies as appropriate. IgG, IgM and IgA anti-VWF: by ELISA (purified VWF coated 1 µg mL⁻¹, anti-Human IgG, IgM and IgA peroxidase labeled antibodies; cut-off < 100%).

Results:

Case A: 11 year-old boy, VWD type 3, on LTP (Haemate-P) due to recurrent hemarthrosis. In August 2014 he presented profuse epistaxis and gum bleeding for more than 7 days in spite of therapy. Laboratory tests showed: VWF:Ag 2% and VWF:RCO 2% without correction on mixing studies and a high level of IgM anti-VWF (350%). Bleedings were treated on demand with higher dose of Haemate-P as well as rFVIIa. After 2 months, symptoms disappeared, and VWF corrected on mixing assays. The title of IgM anti-VWF declined (246%).

Case B: 3 year-old girl, VWD type 2M (FVIII:C 35%, VWF:Ag 44%, VWF:RCO 7%, VWF:CB 11%, E1549K heterozygous mutation), on LTP with Haemate-P once a week due to recurrent severe epistaxis. After 2 months, she presented several nose bleeds, 24–28 hs after Haemate-P without local causes. Laboratory tests showed: VWF:Ag 6% and VWF:RCO 3% without correction on mixing studies. IgM anti-VWF was 233%. Bleeding episodes were treated on demand with Haemate-P. To date, she remains free of bleeding and laboratory parameters returned to basal state.

No symptoms of anaphylaxis associated to VWF concentrates were observed.

Conclusion: We describe two severe VWD cases occurring in children on LTP. The role of free circulating antibodies to VWF is unclear, but the regular testing for neutralizing antibodies should be done in these patients to manage the bleeding episodes. The appearance of inhibitors is not restricted to VWD type 3.

Disclosure of Interest: None declared.

PO647-TUE

von Willebrand disease: the phenotypic characterization in ThailandRuchutrakool T¹, Suwanawiboon B¹ and Nakkinkun Y¹ Charoenkwan P², Nawarawong W³, Sasanakul W⁴ and Chuansumrit A⁴¹Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok; ²Pediatrics; ³Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai; ⁴Pediatrics, Faculty of Medicine Ramathibodi hospital, Mahidol University, Bangkok, Thailand**Background:** von Willebrand disease (vWD) is the most common hereditary bleeding disorder. Because of the variation in the severity of bleeding and the influence of age, blood group and inflammation on von Willebrand factor (vWF) level, the diagnosis of vWD, especially mild form, may be difficult.**Aims:** To figure out the phenotype and laboratory result of Thai patients with vWD.**Methods:** Patients with either previously diagnosed or suspected vWD were enrolled. Data including age at first presentation, familial history, bleeding score and blood group were collected. vWF:Ag, vWF:Rco, vWF:CB, vWF:FVIIIb, vWF:multimer and FVIII:C were performed.**Results:** During the year 2010 to 2015, 53 patients (71% women) were diagnosed with vWD. Twenty two, 19 and 12 patients were type 1, 2A and 3 vWD, respectively. The median age at first bleeding episode of type 1, 2A and 3 vWD was 12, 5 and 3, respectively. Half of the patients had familial history of bleeding problems. There was no significant difference in blood group among individual type. Mean bleeding score of type 1, 2A and 3 vWD were 4.4, 6.6 and 11.3, respectively, (*P*-value 0.026 type 1 VS. 3). Bleeding manifestations were hypermenorrhea (63%), epistaxis (60%), ecchymosis (50%), bleeding from oral cavity (35%) and wound bleeding (26%). The mean value of FVIII:C and vWF:Ag of type 1, 2A and 3 vWD were 52, 48 and 4.7 IU dL⁻¹ and 40, 58 and 3.3 IU dL⁻¹. All patients with type 3 vWD had FVIII:C, vWF:Ag and vWF:Rco of lower than 5 IU dL⁻¹. The mean value of vWF:Rco/vWF:Ag ratio of type 1, 2A and 3 vWD were 0.90, 0.30 and 0.85 (*P*-value 0.0002 type 1 vs. 2A, *P*-value 0.0067 type 3 vs. 2A). The mean value of vWF:CB and vWF:FVIIIb of type 1, 2A and 3 vWD were 44.6, 39.7 and 3.0 IU dL⁻¹ and 55.4, 43.0 and 12.6 IU dL⁻¹.**Conclusion:** For the diagnosis of vWD, bleeding symptoms and familial history should be assessed. A panel of tests regarding vWF can be helpful. Sophisticate tests such as vWF:propeptide and genetic testing should be developed to ascertain some particular types of diseases.**Disclosure of Interest:** None declared.

PO648-TUE

Acquired von Willebrand syndrome in patients with essential thrombocythemiaBykowska K¹, Mendek-Czajkowska E², Sokołowska B³, Wiszniewski A⁴, Baran B¹ and Windyga J⁵¹Department of Hemostasis and Metabolic Disorders;²Department of Hematology, Institute of Hematology and Transfusion Medicine, Warsaw; ³Department of Hematooncology, Medical University, Lublin; ⁴Department of Vascular Surgery; ⁵Department of Disorders of Hemostasis and Internal Medicine, Institute of Hematology and Transfusion Medicine, Warsaw, Poland**Background:** Acquired von Willebrand syndrome (AVWS) is a bleeding disorder. The clinical manifestation and laboratory features resemble congenital von Willebrand disease (VWD). About 10% of AVWS

is associated with myeloproliferative disorders, such as among others essential thrombocythemia (ET).

Aims: Our objective was to establish the frequency of AVWS in ET and correlation between AVWS and bleeding tendency in ET patients.**Methods:** The study comprised 88 patients with newly diagnosed ET. The following tests were performed: ristocetin cofactor (vWF:RCo, Siemens), von Willebrand antigen (VWF:Ag, Siemens), collagen binding (Technoclone), multimers studies (Krizek et al. 2000). The inherited thrombotic risk factors (factor V Leiden, prothrombin G20210A and C677T MTHFR mutation) were detected by PCR/RFLP and ADAMTS13 activity by Technoclone kit.**Results:** AVWS was detected in 27/88 patients with ET with platelet count 1028 ± 262 G/L and WBC 8.44 ± 2.73. In this group: mutation JAK2 (V617F) was present in 19/27 (70%), prothrombin G20210A in 2, homozygous MTHFR C677T in 2 and in 1 person Leiden mutation; laboratory evaluation of VWF parameters showed: VWF:RCo 22–48%; VWF:CB 24–54%; VIII:C 44–102%; VWF:Ag 49–140. VWF:RCo/VWF:Ag was < 0.5 in 11/27 person and > 0.7 in 4/27. Patients without AVWS (61/88) were comparable to persons with AVWS in: age, blood group distribution, WBC amount and ADAMTS13 activity. They differed in platelet count: 863.8 ± 297 G/L and prevalence of mutations. JAK2 (V617F) was present in 27/61 (44.2%), G20210A prothrombin in 1/61 and homozygous MTHFR (C677T) in 7/61 patients. The mean of VWF:RCo, VWF:CB and VWF:Ag were within normal range although the rate of VWF:RCo was < 0.6 in 22.8% of patients (71.4). Clinical evaluation showed no difference in prevalence of thrombotic events. The bleeds occurred in 3/27 patients with AVWS.**Conclusion:** In patients with ET the only parameter which correlated with AVWS appearance was the higher platelet level.**Disclosure of Interest:** None declared.

PO649-TUE

Identification of novel vwf gene mutations in the Turkish type 2 VWD patient populationBerber E¹, Oymak Y², Baltaci OF³, Diz-Küçükaya R⁴, Zulfikar BO⁵ and Kavakli K⁶¹Molecular Biology and Genetics, Istanbul Arel University, Istanbul; ²Pediatric Hematology, Izmir Behcet Uz Children Hospital, Izmir; ³Molecular Biology and Genetics, Istanbul technical University; ⁴Hematology, Istanbul Bilim University, Istanbul; ⁵Pediatric Hematology, Istanbul University, Istanbul; ⁶Pediatric Hematology, Ege University, Izmir, Turkey**Background:** The functional deficiency of von Willebrand Factor (VWF) causes Type 2 von Willebrand Disease (VWD) which is classified as 2A, 2B, and 2M based on defective interaction with platelets, and as 2N based on defective binding to the FVIII molecule. VWF:RCo/VWF:Ag ratio is < 0.6 in 2A, 2B and 2M patients. Mutations causing Type 2 VWD are in exon 17 to 25 and exon 28 of the VWF gene.**Aims:** To screen the VWF gene of Type 2 VWD patients with VWF:RCo/VWF:Ag level < 0.6 to genetically diagnose the Type2 subtypes.**Methods:** Fifty patients were informed, consented in accordance with the Declaration of Helsinki. The VWF gene exon 17 to 25 and 28 were analyzed by Direct DNA sequencing analysis.**Results:** Two patients were homozygotes for previously known Type 2N (c.2446C>T) and Type 3 VWD (c.3108 + 5 G>A) mutations. Three novel variations were identified in three patients: one was heterozygous for Int18 + 166delCTCCTCCCT and Int18 + 175InsGAGCT, the other was heterozygous for c.C4967T change in exon 28 and the third was heterozygous for TGC insertion between c.4876 and c.4877. 13 of the patients were compound heterozygous and 13 of the patients were compound homozygous for c.A4141G and c.T4641C polymorphisms.

Conclusion: In conclusion, this study revealed presence of novel VWF gene mutations in addition to the previously known mutations in the patient population. One of the patients was diagnosed as Type 2N patient and one patient was rediagnosed as Type 3 patient. In silico analysis of the novel deletion and insertion mutation in Intron 18 with splice site prediction revealed that it might affect the splicing by creating an additional donor site. c.C4967T causes p.Thr1656Met substitution, TGC insertion at c4876-c.4877 causes insertion of Leu at p.1541Leu-1542Glu in the VWF A2 domain. Polyphen analysis of both changes revealed that they are damaging. c.A441G and c.T4641C polymorphisms were identified with 39% allele frequency. Future studies will reveal whether these two polymorphisms produce a haplo-type associated with VWD.

Disclosure of Interest: None declared.

PO650-TUE

Cancers in patients with von Willebrand disease: a survey from the Italian association of hemophilia centres

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Background: Besides its role in hemostasis, von Willebrand factor (VWF) seems to have an antitumoral effect through a negative modulation of angiogenesis and induction of apoptosis.

Aims: To elucidate the clinical significance of this role, we have done a retrospective study on cancers among Italian von Willebrand disease (VWD) patients on behalf the Italian Association of Hemophilia Centres (AICE).

Methods: A questionnaire with demographic, clinical, and treatment data of VWD patients with cancer was sent to the 54 Italian Haemophilia Treatment Centres (HTCs).

Results: Eighteen HTCs (33%) provided information on 92 VWD patients (61 alive/31 deceased) with 106 cancers collected during the period 1981-June 2014. Of them, 19 (18%) were hematological and 87 (82%) solid cancers (including 6 hepatocellular carcinoma detected in 6 HCV+ patients). At diagnosis, 25% of cancers were metastasized. Median age at cancer diagnosis was 58 y (21–85), while at death 68 y (21–84). Cancer progression was the principal cause of death (77% cases) followed by bleeding and infectious complications (10% and 13%). Fifty-six patients (61%) had type 1, 33 (36%) type 2 and 3 (3%) type 3 VWD; this distribution was quite different from that observed in the whole VWD population followed at the 18 HTCs (79% type 1, 16% type 2 and 5% type 3; type 2 vs. non-type 2 distribution between cancer group and whole VWD population: $P < 0.001$; OR 2.91; 95% CI: 1.79–4.64). Patients underwent 52 invasive and 72 surgical procedures treated with VWF/FVIII concentrates in 77 cases, desmopressin alone in 24 cases and both in 7 cases. Hemorrhagic complications were

observed rarely (2% of invasive procedures and radiotherapy, 6% of surgeries).

Conclusion: Our data document a substantial number of cancers among VWD patients and a safe management by HTCs physicians. Particularly interesting is the finding of the inversion of the ratio among VWD types, being VWD type 2 significantly more frequent in our cohort than in the whole VWD population.

Disclosure of Interest: None declared.

PO651-TUE

Modifications of hemostatic therapy for patients with von Willebrand disease based on comorbidity

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Background: Due to improving of medical care for patients (pts) with von Willebrand's disease (VWD) nowadays the pts life expectancy and comorbidities are increased.

Aims: To optimize hemostatic therapy with factor VIII/von Willebrand factor (VIII/VWF) concentrates for pts with VWD based on comorbidity.

Methods: Twenty VWD-pts were treated with VIII/VWF: 10 pts - with type 1 (50%), 2 pts - type 2 (10%), 8 pts - type 3 (40%). The middle age was 40 (25–71) years. The gender distribution male/female was 7/13. In 50% of pts there were 3–4 bleeding episodes per month. For hemostasis were used 2 VIII/VWF concentrates with ratio 2.4/1 and 0.9/1. Pharmacokinetic studies were conducted during 48 h. Screening for diagnostic of comorbidities was done for everybody.

Results: In 15 pts (75%) the comorbid diseases were detected: gastrointestinal – in 4 pts (20%), oncology – in 2 pts (10%), autoimmune – in 2 pts (10%), cardiovascular – in 6 pts (30%), neurological in 1 pts (5%). 3 pts (15%) with recurrent gastrointestinal bleedings were on prophylaxis with 40 IU kg⁻¹ bw twice per week, hemorrhagic syndrome was absent during the year. 17 pts (80%) received on demand treatment with average dose 25 IU kg⁻¹ bw in the total group. Considering the probability of thrombotic complications not for surgery average dose in pts with cancer and cardiovascular diseases was 15 ± 5 IU kg⁻¹ bw, in pts with neurological pathology – 35 IU kg⁻¹ bw, autoimmune diseases – 25 IU kg⁻¹ bw. For 60 year-old type 2 VWD-pt with cirrhosis, ischemia and atrial fibrillation 5 teeth removal was carried out. Loading dose of VIII/VWF concentrate 0.9/1 was 70 IU/kg bw with maintenance dose 25 IU/kg bw within 3 days for the extraction of 3 teeth and then 36/18 (within 4 days) IU/kg bw of VIII/VWF concentrate 2.4/1 for the extraction of the next 2 teeth. There were no complications. 75% FVIII level was maintained within 8 days.

Conclusion: Personalization of hemostatic therapy avoids complications in pts with VWD and comorbidity.

Disclosure of Interest: None declared.

PO652-TUE

Acquired von Willebrand syndrome (AVWS) with a type 2B phenotype secondary to an IGA autoantibody with anti-von Willebrand factor activity

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Background: AVWS is a rare bleeding disorder and usually mimics von Willebrand disease type 1 or 2A and exceptionally 2B.

Aims: To report a case of woman with AVWS with a 2B phenotype misdiagnosed as ITP.

Methods: Platelet count (PC); FVIII:C; VWF:Ag; VWF:RCO; VWF multimers; VWF 2B/GPIb genotypic analysis; RIPA at 0.6 mg mL^{-1} , mixing studies with platelet rich plasma (PRP), washed platelets, poor platelet plasma (PPP), IgG depleted plasma and purified VWF. IgG, IgM and IgA anti-VWF (ELISA).

Results: A 41-year-old woman with ITP under corticosteroids was referred to investigate the persistence of bleeding in spite of moderate thrombocytopenia. Laboratory showed (2010) PC $88 \times 10^9 \text{ L}^{-1}$, macrothrombocytopenia, FVIII 80%, VWF:Ag 50%, VWF:RCO < 10% (VWF:RCO/VWF:Ag ratio < 0.7), enhanced RIPA and absence of high and intermediate molecular weight multimers. Genotypic analysis was negative. The patient continued with corticosteroids and tranexamic acid was added. Compliance to treatment was irregular. On 2011 she presented gum bleeding and menorrhagia (PC $23 \times 10^9 \text{ L}^{-1}$, VWF:RCO/VWF:Ag ratio < 0.7). She received corticosteroids plus dapsone and blood transfusion was necessary. On 2013 the patient developed diabetes. Corticosteroids were suppressed and romiplostin once a week was started. Bleedings ended when normal PC was achieved. Additional tests (mixing studies with PRP, washed platelets, PPP, IgG depleted plasma and purified VWF) allowed us to conclude that the patient's PPP contained an activity (no IgG) capable of inducing platelet agglutination of normal washed platelets in presence of purified VWF. Serum immunofixation showed a low concentration IgA-Lambda monoclonal component. Finally the presence of IgA anti-VWF was shown.

Conclusion: Our findings suggest the presence of an autoantibody IgA enhancing VWF binding to platelet GP 1b resulting in type 2B-like AVWS. This case highlights the difficulties in reaching a diagnosis and in the management of bleeding in a patient with an atypical presentation of AVWS.

Disclosure of Interest: None declared.

PO653-TUE

Acquired von Willebrand syndrome in patients treated with left ventricular assist devices

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Background: von Willebrand Factor (vWF), a critical factor in hemostasis, is produced as a huge multimeric plasma protein, and is shear stress-dependently cleaved into a molecular weight of 500–20,000 kDa. Given that large multimers of vWF play critical roles in hemostasis, non-physiological high shear causes excess cleavage of it and bleeding tendency known as acquired von Willebrand syndrome (AvWS), characterized by lack of large multimer of vWF. It has recently been noted that AvWS occurs in patients treated with mechanical circulatory support such as left ventricular assist devices (LVAD).

Aims: To elucidate the relationship between bleeding complication and vWF multimeric pattern in patients treated with LVAD artificial heart.

Methods: We analyzed the multimeric patterns of vWF in 5 Japanese patients recently treated with LVAD.

Results: All 5 LVAD recipients presented AvWS hematologically, demonstrated by reduced or absent the large vWF multimer levels. The first LVAD patient exhibited bleeding in gastrointestinal and respiratory tract, the second LVAD patient presented severe anemia. The other 3 patients under LVAD exhibited no bleeding events although the vWF large multimer levels were reduced. AvWS was most likely caused by LVAD since one patient exhibited vWF normal

pattern before LVAD treatment and another patients did it after heart transplantation.

Conclusion: Hematological AvWS might occur with unexpectedly high prevalence in Japanese patients under treatment with LVAD although not all patients exhibited bleedings.

Disclosure of Interest: None declared.

PO654-TUE

Dental invasive procedures in von Willebrand disease (VWD) outpatients treated with high purity VWF/FVIII complex concentrate: experience of a single center

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Background: Dental procedures in von Willebrand Disease (VWD) patients are associated with risk of bleeding. VWF/FVIII concentrate is the treatment of choice in VWD type 3 and type 2b, but is indicated in VWD subtypes when there is no response to Desmopressin

Aims: To describe dental procedures performed in VWD outpatients utilizing systemic prophylactic therapy with a high purity VWF/FVIII

Methods: In 7 years we performed 31 dental procedures on 12 patients (5M, 7F) affected by VWD: 4 type 1, 4 type 2b, 4 type 3. We treated all patients with high purity VWF/FVIII concentrate

Results: VWD type 1. One patient underwent 3 extractions, other 2 underwent 2 extractions each. Another one underwent 1 scaling, 1 biopsy. They had treated with high purity VWF/FVIII concentrate according to the scheme: 1 h before surgery (t_0), 60 IU kg^{-1} ; from the 12th to 48th hour ($t_{12 \text{ h} \rightarrow t_{48 \text{ h}}}$) after surgery, 30 IU kg^{-1} every 12 h; from the 3rd to 7th post-operative day, $30 \text{ IU kg}^{-1} \text{ day}^{-1}$. VWD type 2b. One patient underwent 5 extractions with VWF/FVIII $50 \text{ IU kg}^{-1} t_0$; $t_{12 \text{ h} \rightarrow t_{60 \text{ h}}}$ 25 IU kg^{-1} , every 12 h, then 25 IU kg^{-1} every 24 h, until 7th post-operative day. One other underwent 1 extraction while treated with VWF/FVIII $50 \text{ IU kg}^{-1} t_0, t_{24}$. One other patient underwent 1 extraction with VWF/FVIII $60 \text{ IU kg}^{-1} t_0 \rightarrow t_{72 \text{ h}}$, 30 IU kg^{-1} in the 4th day. The last one underwent 2 extractions, 1 scaling, 1 biopsy under the administration of VWF/FVIII $30 \text{ IU kg}^{-1} t_0$; then tranexamic acid orally, $40 \text{ mg kg}^{-1} \text{ day}^{-1}$ until 7th post-operative day. VWD type 3. One patient underwent 2 extractions according to the scheme: VWF/FVIII $50 \text{ IU kg}^{-1} t_0$; $t_{12 \text{ h} \rightarrow t_{48 \text{ h}}}$ 30 IU kg^{-1} every 24 h, then tranexamic acid orally, $40 \text{ mg kg}^{-1} \text{ day}^{-1}$ until 7th post-operative day. One other underwent 6 extractions, 1 scaling and another one underwent 1 extraction; they were treated with VWF/FVIII $60 \text{ IU kg}^{-1} t_0$; $t_{12 \text{ h} \rightarrow t_{48 \text{ h}}}$ 50 IU kg^{-1} every 24 h, then $30 \text{ IU kg}^{-1} \text{ day}^{-1}$ until 7th post-operative. The last one underwent 1 scaling with VWF/FVIII $50 \text{ IU kg}^{-1} t_0, t_{24}$. We didn't observe any hemorrhagic or local abscesses

Conclusion: A tailored prophylactic treatment with high purity VWF/FVIII concentrate ensures a good hemostasis

Disclosure of Interest: None declared.

PO655-TUE

Recognition and correlation of a random patient-specific error of the automated von Willebrand factor activity latex immunoassay

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Background: Laboratory diagnosis of von Willebrand disease (VWD) requires measurement of von Willebrand factor (VWF) antigen (Ag)

and activity. The reference VWF activity test is the ristocetin cofactor assay (RCo), but an automated latex immunoassay (Lx) is gradually being accepted as an alternative method. After our implementation of the Lx assay (JTH. 2011; 9:1993–2002), an increased number of samples exhibiting elevated VWF:Lx/Ag ratios (≥ 1.35) was observed with a new lot. The prevalence of samples with elevated ratios was 0.2% during validation ($n = 492$), 1.5% ($n = 4009$) pre-new lot, and 4.8% ($n = 2382$) post-new lot. Correlation of affected samples (ratio ≥ 1.35) with VWF:RCo assay confirmed the suspicion that the VWF:Lx assay was overestimating VWF activity in a random fashion.

Aims: Collaborate with the manufacturer to identify and resolve the root cause of aberrant VWF:Lx results.

Methods: Blinded samples ($n = 45$) were shared with the manufacturer for confirmation of our observation. After identifying the root cause, a new lot VWF:Lx reagent was provided to our laboratory for repeat VWF:Lx testing (106 affected and 246 unaffected samples, ratio < 1.35). Routine statistical analyses were performed.

Results: Presence of bovine IgG, identified in the reagent, was cross reacting with human anti-bovine IgG antibodies (HABIA) in patient samples causing random erroneous VWF:Lx results. Manufacture of a new reagent lot resolved this interference. With the new reagent, 96% of the affected samples' ratios corrected to < 1.35 (t-test, $P < 0.0001$); linear regression of VWF:Lx and RCo, showed better agreement on new lot ($R^2 = 0.90$; y-int 16%) than affected lot ($R^2 = 0.57$; y-int 36%). VWF ratios of the unaffected samples remained essentially unchanged (t-test, $P = 0.07$).

Conclusion: HABIA can result in random errors with the VWF:Lx assay which, once recognized, has been remedied. Our experience emphasizes the importance of correlation of VWF laboratory data and manufacturer communication/collaboration in pursuit of aberrant results.

Disclosure of Interest: None declared.

Von Willebrand factor II

PO656-TUE

Long term exercise significantly decreases von Willebrand factor antigen levels in abdominally obese adults

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Background: Elevated von Willebrand Factor antigen (VWF:Ag) levels are observed in obesity and metabolic syndrome. However, the impact of duration and intensity of exercise on VWF levels in individuals with abdominal obesity is unknown.

Aims: This study examines the effects of exercise amount and intensity on plasma VWF in an inactive obese cohort. Additionally, VWF association to changes in metabolic syndrome criteria in response to exercise are examined.

Methods: A 24-week, parallel group exercise trial was performed with 217 abdominally obese adults approved by Queen's University Health Sciences Research Ethics Board. Participants were randomized to: C: no-exercise control ($N = 49$), or 5 sessions per week of exercise. LALI: low amount, low intensity exercise (Females (F): 180 kcals/session, males (M): 300 kcals/session @ 50% $\text{VO}_{2\text{peak}}$, $N = 55$), HALI: high amount, low intensity (F: 360 kcals/session, M: 600 kcals/session for men @ 50% $\text{VO}_{2\text{peak}}$, $N = 59$), HAHl: high amount, high intensity (F: 360 kcals/session, M: 600 kcals/session @ 75% $\text{VO}_{2\text{peak}}$, $N = 45$). At weeks 0, 16, and 24, waist circumference, body mass index (BMI), cardiorespiratory fitness, glucose tolerance, lipids, VWF:Ag, and VWF propeptide (VWFpp) were assayed.

Results: At week 16, HAHl and HALI VWF:Ag significantly decreased from week 0 (HAHl: $-8.4 \pm 25 \text{ U dL}^{-1} \pm \text{SD}$, $P = 0.004$,

student's t-test; HALI: $-9 \pm 22 \text{ U dL}^{-1}$, $P = 0.003$), although LALI was not significantly lower (0.7 ± 21.9 , NS) compared to C (8 ± 27). VWF:Ag further decreased at 24 weeks for HAHl (-12.8 ± 20.8 , $P = 0.003$) and HALI (-12.7 ± 18.8 , $P = 0.001$), LALI was not (-5 ± 24.4 , NS) compared to C (1 ± 24.1). This did not correlate with age or gender. VWFpp was not significantly changed.

Conclusion: In response to high amounts of exercise, VWF antigen levels were significantly lowered compared to inactive control. This along with unchanged VWFpp suggests a decrease in VWF:Ag half life. This study demonstrates that exercise can improve elevated VWF levels in metabolic syndrome.

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PO657-TUE

Development of a VWF propeptide assay

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Background: von Willebrand disease (VWD) is the most common bleeding disorder in the world. VWD patients are largely under-diagnosed or misdiagnosed. About 80% of VWD patients are diagnosed with type 1 VWD and about fifty percent of them present with increased clearance of VWF. The ratio between von the Willebrand factor propeptide (VWFpp) and the mature VWF antigen (VWF: Ag) can be used to diagnose these patients. The current commercially available assays for VWF propeptide are extremely expensive.

Aims: The aim of this study was to develop an ELISA assay to determine the plasma levels of the VWF-propeptide.

Methods: Antibody fragments were selected against the VWFpp by using phage display technology. The VWFpp was first displayed on yeast, since no commercial preparation of the VWFpp exists. By using phage display technology, we selected two single chain variable antibody fragments (ScFv) from almost two-hundred phage colonies that bind specific to the VWFpp and not to the yeast on which it was displayed. The antibody fragments were then purified on protein A columns and tested for specific binding to the VWFpp.

Results: The purified ScFv were able to detect VWFpp in normal plasma. By comparing our assay to commercial assays, our antibody fragments showed a higher binding affinity for VWFpp in plasma at especially lower plasma concentrations than an assay using commercial antibodies to the VWFpp. Our assay is also more cost-effective than commercial antibodies, since it was not necessary to use expensive infrastructure as in the case with the development of antibodies using experimental animals.

Conclusion: The combination of yeast- and phage display could be the reason why the 2 ScFv were selected successfully without alterations in specificity as both technologies are known to produce antibodies with the highest binding affinity. The next step is to validate our assay and possibly commercialise it as a cost-effective assay for the determination of VWFpp in plasma.

Disclosure of Interest: None declared.

PO658-TUE

Cross-species inhibition of human and murine von Willebrand factor by a single domain VHH-antibody

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Background: Monoclonal antibodies against human von Willebrand factor (hVWF) have contributed to decipher the roles of the different functional domains of VWF. However, few antibodies display cross-reactivity with murine VWF (mVWF), thereby limiting their application in *in vivo* murine models. Llama-derived single domain antibodies (VHH or nanobodies) have become a potential alternative for murine-derived monoclonal antibodies.

Aims: To generate VHH-antibodies that cross-react with hVWF and mVWF.

Methods: A llama was immunized via repeated and alternated injections with purified hVWF and mVWF. VHH-antibody libraries were screened against hVWF using phage-display technology.

Results: A first screening resulted in 13 unique VHH-antibodies recognizing different domains of hVWF. Among cross-species reacting VHH-antibodies, one (designated KB-VWF-006) recognized the VWF A1 domain with high affinity (K_d, app=1.1 pM). Avidity was enhanced > 200-fold via dimerization of KB-VWF-006 (KB-VWF-006-bi; K_d, app=4.2 fM). KB-VWF-006-bi efficiently inhibited ristocetin-induced platelet aggregation (RIPA; IC₅₀ 60 nM) using platelet-rich plasma, while leaving ADP-, TRAP- or collagen-induced aggregation unaffected. Similarly, KB-VWF-006-bi (130 nM) fully inhibited platelet adhesion to a collagen-matrix in high-shear perfusion assays. Interestingly, KB-VWF-006-bi was similarly efficient in inhibiting hVWF- and mVWF-induced RIPA using isolated platelets and purified VWF (IC₅₀ = 8 nM and 5 nM, respectively), confirming its cross-species activity. *In vivo* activity of KB-VWF-006-bi was evaluated in a tail-clip bleeding model 5 min following intravenous injection (1–25 mg kg⁻¹; n = 23) in wild-type C57B6 mice. KB-VWF-006-bi induced bleeding dose-dependently (EC₅₀ 5.2 ± 0.7 mg kg⁻¹), showing that this antibody is functional *in vivo*.

Conclusion: KB-VWF-006-bi inhibits VWF function *in vitro* and *in vivo*. VHH-antibody technology seems an appropriate tool to specifically select cross-species reacting antibodies.

Disclosure of Interest: None declared.

PO659-TUE

High activity of von Willebrand factor and levels of VWF-cleaving protease (ADAMTS-13) in term and preterm neonates

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Background: Von Willebrand Factor (VWF) has a central role in primary hemostasis, platelet adhesion and aggregation. Its biological activity is related to the size of VWF multimers, which spontaneously bind to platelets and induce formation of microthrombi in the circulation. This mechanism is downregulated by the plasma protease ADAMTS-13 (A Disintegrin and Metalloprotease with Thrombospondin motif). Low levels of ADAMTS-13 are mainly been reported in patients with thrombotic thrombocytopenic purpura (TTP). Only few

studies have addressed the levels of ADAMTS-13 in neonates and pre-term infants

Aims: The aim of our study was to investigate ADAMTS-13, VWF antigen and Ristocetin cofactor activity in neonates, evaluating potential correlations and risk for perinatal complications.

Methods: Cord blood samples were taken from the umbilical cord of neonates immediately after clamping. ADAMTS 13, VWF:Ag and VWF: RCoF% were assessed by standard measurements. Demographic data and clinical details were recorded.

Results: Levels of ADAMTS-13 were found to be significantly higher in preterm babies (Median 72.0 IQR 34.8,) in comparison to full term (Median 57.5 IQR 23.8) and adult controls (Median 54.0 IQR 19.5) (*P* < .05). Conversely, a significant elevation of VWF antigen was observed in preterm and term infants compared to adults (*P* = 0.014). Levels of VWF:Ag% were found to be highest in full term infants and lowest in adults (*P* < .05). Levels of RiCoF were similar in preterm and full term but higher than in adults (*P* < .05). IUGR, maternal hypertension and fetal distress or presence of NEC/ROP affected ADAMTS-13, VWF; Ag and VWF:Rcof% compared to infants without these complications

Conclusion: The levels of ADAMTS-13 among neonates were lower as compared to healthy adult. These associations with the risk of prematurity complications deserve further attention.

Disclosure of Interest: None declared.

PO660-TUE

Angiogenic characteristics of blood outgrowth endothelial cells from patients with von Willebrand disease

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Background: Endothelial von Willebrand factor (VWF) has been shown to negatively regulate angiogenesis, the formation of new vessels from pre-existing ones. Blood Outgrowth Endothelial Cells (BOECs) isolated from type 1 and 2 von Willebrand disease (VWD) patients showed enhanced *in vitro* angiogenesis compared with healthy control BOECs. Characterisation of the angiogenic response of BOECs from VWD patients is however limited and differences between the different types of VWD have not been investigated in detail.

Aims: The aim of this study was to further explore the potential pathogenic role of VWF mutations in regulating angiogenesis.

Methods: We isolated BOECs from 4 healthy individuals, 4 patients with type 1, 5 patients with type 2, 1 patient with type 3 VWD and 1 heterozygous carrier of a type 2N mutation. Angiogenic response was assessed by cell migration using a scratch-wound assay and time-lapse microscopy and tube formation using an *in vitro* Matrigel assay.

Results: Migration velocity and total tube formation was similar between BOECs from VWD patients overall and control BOECs. BOECs from the type 3 VWD patient and one type 2B patient showed increased migratory velocity and tube formation compared with BOECs from other patients and controls. Migratory directionality was impaired in 8 out of 10 VWD BOECs. The ability to form tubes was limited to early passages of VWD BOECs, but not for control BOECs.

Conclusion: We did not observe an increased angiogenic potential in VWD BOECs compared with control BOECs, but individual patients showed both increased and decreased angiogenic potential. Interestingly, almost all patients showed impaired migratory directionality.

BOECs from VWD patients provide an easily accessible model to investigate the pathogenic role of VWF in regulating angiogenesis in VWD. However, the loss in angiogenic phenotype at later passages should be taken into account when performing angiogenesis assays with BOECs from VWD patients.

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PO661-TUE

Regulation of VWF-GPIB interaction with modified recombinant botrocetin

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Background: Botrocetin is a hetero-dimer protein of α and β subunits purified from snake venom of *Bothrops jararaca*. Botrocetin specifically binds to von Willebrand factor (VWF), and the complex induces GPIIb-dependent platelet agglutination. We have cloned cDNAs of botrocetin-like protein (Bot-2) and obtained the recombinant proteins (rBot-2).

Aims: We have tried to identify the important residues of Bot-2 for binding to VWF and platelet GPIIb, and tried to produce unique mutant rBot-2 that binds to VWF but interferes GPIIb-binding.

Methods: Point mutation was introduced into cDNAs of Bot-2 subunits by QuickChange II site-directed mutagenesis kit. Mutated cDNAs were introduced into 293T cells with GeneJuice transfection reagent and each recombinant protein in cultured medium was purified by anti-botrocetin monoclonal antibody-conjugated agarose column. Binding activities of rBot-2s for VWF or GPIIb were examined by ELISA. Effects of rBot-2s on ristocetin- or shear-induced platelet agglutination were assessed.

Results: The mutant rBot-2 substituted to Ala at D70 in β subunit showed decreased binding activity toward immobilized VWF. The mutant of R115A and K117A in β subunit after complexed with VWF has decreased binding activities toward GPIIb (glycocalicin). We have focused on the GPIIb-binding activity of VWF-Bot-2 complex and tried to produce rBot-2 substituted to acidic residues at R115 and K117. The rBot-2 containing both R115E and K117E substitution had normal VWF-binding activity but showed no apparent platelet agglutinating activity. To the contrary, this double mutated protein inhibited ristocetin- or shear-induced platelet agglutination.

Conclusion: We concluded that D70 in β subunit of botrocetin is important for VWF-binding, and that R115 and K117 in β subunit are essential for interacting with GPIIb. Double mutated rBot-2 containing R115E and K117E substitution has a potential activity leading to an anti-thrombotic reagent.

Disclosure of Interest: None declared.

PO662-TUE

von Willebrand factor to monitor percutaneous aortic valve interventions

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Background: Percutaneous aortic valve procedures are a major breakthrough in the management of patients with aortic stenosis (AS). In some circumstances their result can still be inadequate while their evaluation in real-time is challenging. We hypothesized that: 1) recovery of high molecular weight multimers (HMWM) of von Willebrand factor (VWF) defect, could occur within minutes following acute changes in blood flow, 2) a bedside point-of-care assay (PFA-CADP), reflecting HMWM changes, could be used to monitor percutaneous aortic valve procedures.

Aims: To investigate the time course of HMWM and PFA-CADP recovery in models and patients with instantaneous reversal of pathological high shear stress.

Methods: We investigated the time course of the recovery of HMWM defects under conditions allowing an almost instantaneous reversion of high shear stress in an AS-rabbit model ($n = 17$). We further investigated the recovery of HMWM and monitored these changes with PFA-CADP in AS-patients undergoing transcatheter aortic valve implantation (TAVI, $n = 20$) or balloon valvuloplasty (BAV, $n = 10$).

Results: Experiments in the AS-rabbit model demonstrated that recovery of HMWM occurs within 5 min. TAVI-patients experienced an acute decrease in shear stress and a recovery of VWF defects within minutes of implantation. In BAV-patients, a modest improvement in shear stress and no recovery of VWF defects were observed. PFA-CADP profiles mimicked HMWM recovery both in TAVI-patients (correction) and BAV-patients (no correction). In TAVI-patients, the PFA-CADP at the end of the procedure was significantly higher in the 4 patients with aortic regurgitation (AR) than in the 16 patients without AR (225 ± 41 s vs. 100 ± 23 s, $P < 0.01$).

Conclusion: These results demonstrate that the recovery of VWF multimeric pattern is highly dynamic, occurring within minutes after changes in blood flow. Assessing VWF changes overtime with a point-of-care assay like PFA-CADP could be useful to monitor acute changes in blood flow during BAV and TAVI procedures.

Disclosure of Interest: None declared.

PO663-TUE

Platelet and endothelial function evaluation in pediatric chronic kidney disease (CKD)

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Background: CKD is classified in stages 1 to 5, according to glomerular filtration rate and is associated with atherosclerosis. VWF levels are a marker for endothelial or platelet function. ADAMTS13 cleaves

VWF, preventing thrombus formation in glomerular capillary lumen. P-selectin/CD62P is a useful marker of platelet activation. Renin-angiotensin-aldosterone system (RAAS) inhibitors reduce vascular and glomerular endothelium injury by inhibiting the pro-fibrotic and pro-inflammatory actions.

Aims: Evaluating platelet and endothelial function through the P-selectin expression, VWF and ADAMTS13 plasma levels determination in CKD pediatric patients.

Methods: This study was approved by local Ethical Committee and consent was obtained in all cases. It included 49 CKD patients (6–18 years old), stages 1 and 2 ($N = 11$) and stage 3 ($N = 38$) and 31 healthy volunteers. Platelet P-selectin expression was evaluated by flow cytometry, and VWF and ADAMTS13 levels by ELISA. Groups were compared considering CKD stages and the use of RAAS inhibitors.

Results: CKD patients showed lower P-selectin comparing to control group [1.735% (CI: 1.238) vs. 2.360% (CI: 1.897), $P = 0.0006$]. VWF levels were higher in CKD patients than in controls [1157 ± 384.8 vs. 843.3 ± 366.9 mU mL⁻¹, $P = 0.0005$]. ADAMTS levels did not differ between CKD patients and controls (1537 ± 481.2 vs. 453.5 ± 1509 ng mL⁻¹). However, they are higher in stages 1 and 2 CKD than in stage 3. Stage 3 patients were compared according the RAAS inhibitors treatment (users, $N = 24$ and non-users, $N = 14$). VWF levels were lower in RAAS users [1063 ± 315.0 vs. 432.1 ± 1429 mU mL⁻¹; $P = 0.0047$]. P-selectin and ADAMTS did not differ among groups.

Conclusion: Our data point to a potential maintenance of ADAMTS13 antithrombotic property in CKD pediatric patients at an early stage. Despite higher VWF, low P-selectin suggests platelet inhibition *in vivo*. The highest VWF found in RAAS inhibitors' non-user patients may indicate endothelial injury and higher thrombotic potential in these patients.

Disclosure of Interest: None declared. Support: CNPq and FAP-EMIG/Brazil.

PO664-TUE

Functional study of two new mutations in the C4 domain of von Willebrand factor

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Background: In the framework of the French National Reference Center for von Willebrand Disease (VWD), two new mutations in the C4 domain have been identified: p.V2517F and p.R2535P. Patients displayed a mild bleeding phenotype and were classified as type I VWD. The C4 domain of von Willebrand factor (VWF) contains the RGD sequence which allows binding to integrin alphaIIb beta3. So far, functional studies about mutations in this domain have been scarce.

Aims: To characterize binding of recombinant VWF proteins carrying these mutations to various ligands of VWF.

Methods: Stable cell lines (BHK-furin cells) producing each mutant were developed. Culture medium was collected and concentrated. Binding tests to monoclonal antibodies (mAbs) to VWF, to collagens type I and 3, to glycoprotein Ib, to factor VIII and to integrin alpha-IIb beta3 were performed using an Elisa setting.

Results: VWF p.V2517F bound normally to all mAbs covering the entire VWF molecule. Binding of this mutant was normal to all ligands except to aIIb beta3 where a 50% inhibition of binding was measured. VWF p.R2535P displayed mildly reduced binding to the different mAbs. However, binding of this mutant to alphaIIb beta3 was reduced

by 90% compared to wild-type VWF. Binding to other ligands was also decreased although to a lesser extent (between 70% of reduction for binding to FVIII to almost normal binding to glycoprotein Ib).

Conclusion: We have identified previously unrecognized VWF mutants with defects in binding to alphaIIb beta3 when the protein was expressed as homozygous mutants. The heterozygous state of the patients will warrant a milder phenotype.

Disclosure of Interest: None declared.

PO665-TUE

POstoperative changes in von Willebrand factor (VWF) after orthopaedic surgery in haemostatically healthy patients

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Background: Defects in VWF may result in bleeding, e.g. postsurgical bleeding events. However, the natural course of VWF after orthopaedic surgery is unknown. These patients are supposed to develop hypercoagulability that encourages thrombotic events.

Aims: The aim of the project was to analyse VWF levels in haemostatically healthy patients undergoing hip- or knee-surgery and their correlation with surgical blood loss.

Methods: In total 109 surgeries in haemostatically healthy patients were performed (hip: $n = 64/59\%$; knee: $n = 45/41\%$) in this prospective study. Blood samples were collected pre- and post-op (0, 4–8 h, 1, 2, 3, 4, 6 post-op-day) to analyse VWF and other coagulation parameters. Furthermore, volume of blood loss was assessed intra- and post-operatively.

Results: Depending on surgery type a continuous increase of VWF-activity was detected in both subgroups and also in the total group from pre- (123.4%) to D3 post-op (261.7%). From D4 post-op the VWF-activity-level decreased in all groups. VWF-antigen levels rose until D4 post-op from 132.7% to 247.7%, respectively. Significant differences in VWF-activity as well as -antigen were found between hip- and knee-surgery from D2 to D6 ($P < .05$) showing higher levels of VWF in hip-surgery. Furthermore, low pre-op VWF clearly led to significantly higher total blood loss ($P < .005$) and secondary post-op blood loss ($P < .05$). In addition, pre-operative FXIII-level did not influence total blood loss.

Conclusion: The course of VWF after surgery showed constantly rising values until day 3 and longterm highly elevated levels postsurgically. The reason and clinical significance for this are unclear. Further studies are necessary to clarify the role of this natural course with regard to bleeding cessation and wound healing.

Disclosure of Interest: None declared.

PO666-TUE

Generation of reference intervals for two automated, new-generation von Willebrand factor activity assays on a large donor population

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Background: Circulating von Willebrand factor (VWF) levels are influenced by a variety of biological factors. Normal individuals with O

blood group demonstrate 25–35% lower VWF levels than non-O individuals. Although assessment of VWF values for von Willebrand disease (VWD) diagnosis tends to be made irrespective of blood group, blood group-specific cut-offs can be useful in some cases.

Aims: To generate general and blood group-specific reference intervals (RIs) for two new-generation VWF activity assays on a large donor population.

Methods: The HemosIL VWF:RCo assay (on an ACL TOP 500) involves binding plasma VWF to recombinant GpIb α fragments attached to latex particles with the aid of ristocetin. The Innovance VWF:GpIbM assay (on a Sysmex CS2000i) utilises a recombinant GpIb α containing two gain-of-function mutations and is ristocetin-independent. VWF antigen (VWF:Ag) was determined using HemosIL VWF:Ag immunoassay on the ACL TOP 500.

Results: Three parameters were derived from each of the 201 plasma samples obtained from healthy donors. 183/201 samples had a known ABO blood group. RIs were calculated as 95% confidence limits. Median (M) and RI values are presented as follows: Non-O blood group ($N = 84$): VWF:Ag: 110.8; 64.5–185.5; VWF:RCo: 97.7; 50.1–153.5; VWF:GpIbM: 100.1; 37.1–164.3. Group O ($N = 95$): VWF:Ag: 82.8; 53.2–127.4; VWF:RCo: 72.3; 44.8–123; VWF:GpIbM: 66.8; 31.6–105.1. Combined results, irrespective of blood group, ($N = 201$): VWF:Ag: 94.2; 53.3–169.7; VWF:RCo: 86.6; 47.0–152.9; VWF:GpIbM: 80.2; 38.0–165.2. All results are expressed in IU dL $^{-1}$.

Conclusion: Blood group significantly influences VWF levels and it is important to use blood group specific reference intervals for the diagnosis of VWD. The VWF activity values also depend on the method of analysis applied. VWF:GpIbM demonstrates lower values for VWF activity when compared with VWF:RCo. Reagent / analyzer specific RIs should be locally determined.

Disclosure of Interest: None declared.

PO667-TUE

Exposure of von Willebrand factor (VWF) to vortex shear significantly enhances its proteolysis by plasmin

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Background: Recent studies show that plasmin acts as a natural alternative to ADAMTS13 in a mouse model of thrombotic thrombocytopenic purpura (TTP). Exposure of VWF to shear by vortexing enhances its proteolysis by ADAMTS13. However, plasmin-mediated proteolysis of VWF has not yet been examined in this quantitative manner.

Aims: In this study, we sought to examine plasmin-mediated proteolysis over time of plasma-derived VWF (pdVWF) under shear, using a modified form of the vortex assay to allow timepoint sampling and multimer analysis.

Methods: Purified pdVWF (6 $\mu\text{g mL}^{-1}$) and plasmin (100 $\mu\text{g mg}^{-1}$ VWF) were vortexed at 2500 rpm for 5 min. Proteolysis was stopped with 15 μM aprotinin. High molecular weight (HMW) and low molecular weight (LMW) VWF multimers were assessed by agarose gel electrophoresis, followed by immunoblotting and densitometric analysis (Image J). All experiments described are $n \geq 3$.

Results: Multimeric pdVWF is cleaved by plasmin in a time- and shear-dependent manner, with loss of HMW pdVWF (0 mins vs. 5 mins, $P = 0.0048$) and gain of LMW pdVWF (0 mins vs. 5 mins, $P < 0.0001$). In the absence of plasmin or in the absence of shear, there is no significant multimeric alteration. Plasmin cleavage of VWF is completely prevented with the known plasmin inhibitors, aprotinin (0.5 μM ; $P = 0.018$), and the lysine analogue ϵ -aminocaproic acid (50 mM; $P = 0.0039$). Shear-dependent plasmin-mediated proteolysis of pdVWF is observed without CaCl_2 ($P = 0.0240$) but is enhanced in its presence (10 mM; $P < 0.0001$). In contrast, CaCl_2 is absolutely

required for ADAMTS13 proteolysis in this assay (no CaCl_2 , $P = 0.7890$; CaCl_2 , $P < 0.0001$). Furthermore, under static conditions, plasmin-mediated proteolysis of VWF is significantly enhanced in the presence of ristocetin (1 mg mL^{-1} ; 60 min; $P = 0.0107$) or urea (1.5 M; 120 min; $P = 0.0067$).

Conclusion: Shear-induced plasmin-mediated proteolysis of VWF may be of relevance in the acquisition of VWD in aortic stenosis or upon left ventricular assist device (LVAD) implantation.

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PO668-TUE

Belgian multicenter study into von Willebrand disease (B-Will study): first results

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Background: von Willebrand Disease (VWD) is an autosomally inherited bleeding disorder caused by a quantitative or qualitative defect of von Willebrand factor (VWF). The current ISTH classification is based on measuring VWF:Ag, VWF:RCo, Ristocetin Induced Platelet Aggregation (RIPA) and VWF multimers, but ignores molecular analysis of the VWF gene.

Aims: Multicenter family-based analysis of VWD in Belgium.

Methods: Blood samples were collected from patients with known VWD (all types) with one of the following characteristics: VWF:Ag $< 35\%$, VWF:RCo $< 35\%$, VWF:RCo/VWF:Ag < 0.7 , VWF:CB/VWF:Ag < 0.7 , FVIII:c/VWF:Ag < 0.5 , or positive low RIPA, and grouped in families each consisting of a proband and affected siblings or parents. FVIII:c, VWF:Ag, VWF:RCo, VWF:CB, VWFpp, VWF-FVIII binding (if indicated), PFA-100 and RIPA, VWF multimers and molecular analysis were performed to (sub) classify the patients.

Results: So far, blood has been collected from 123 patients representing 77 families with VWD. Distribution of different subtypes is as follows: VWD type 1 in 77/123 patients, type 2 in 31/123 (19/123 type 2A, 11/123 type 2M and 1/123 type 2N), and type 3 in 5/123 patients. All cases of type 3 VWD (5/123) were based on the presence of type 1 VWD in both parents. Molecular analysis is still ongoing with currently 56/123 patients fully analyzed and the remainder partially. So far, 27 mutations in the VWF gene have been found in 56/123 patients, of which 7 are new to the ISTH VWD database and awaiting gene expression studies.

Conclusion: This study is the first characterisation of VWD in the Belgian population. These data will contribute to understanding the phenotype-genotype relationship in VWD. Full laboratory and multimeric analysis for all current is finished. Molecular analysis is ongoing. Accrual of patients by participating centers is ongoing to provide a more comprehensive overview of the Belgian VWD population.

Disclosure of Interest: None declared.

PO669-TUE

The von Willebrand factor A2 domain disulfide bond exists in oxidized and reduced states in the circulation and regulates function

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Background: von Willebrand factor (VWF) is a multimeric plasma protein that captures platelets to the injured blood vessel wall during thrombosis, through interactions with vessel wall collagen and platelet receptors GPIb α and α 2b β 3. Only the largest VWF multimers are effective at capturing platelets in the high shear of flowing blood and multimer size is regulated by proteolysis of the A2 domain by ADAM-TS13. The VWF A2 domain contains a single disulfide bond that links adjacent cysteine residues, Cys1669 and Cys1670.

Aims: The Cys1669-Cys1670 bond has features of an allosteric disulfide, suggesting that it may act as a functional switch in VWF.

Methods: VWF was isolated from human plasmas and the redox state of the Cys1669-Cys1670 disulfide bond was determined by differential cysteine alkylation and mass spectrometry.

Results: The A2 domain disulfide exists in oxidized and reduced states in circulating VWF in healthy donors ($n = 33$; 12 males, 21 females; 31–82 years old; mean of 68% of the Cys1669-Cys1670 disulfide is reduced, range 48% to 81%). The extent of reduction of circulating VWF positively correlates with blood platelet number ($P < 0.0001$) and negatively correlates with neutrophil number ($P < 0.005$), indicating a cell-based redox control of the disulfide. Molecular Dynamics simulations reveal that reduction of the disulfide has a pronounced effect on A2 domain dynamics and structure, suggesting that the different redox states have different properties. Indeed, reduced VWF is more efficiently cleaved by ADAMTS13 than the oxidized protein. Notably, compared with healthy donors, the VWF disulfide is significantly more oxidized in cardiovascular at-risk patients ($P < 0.0001$).

Conclusion: The VWF A2 domain disulfide bond exists in oxidized and reduced states in the circulation and elevated oxidized levels are associated with impaired hemostasis.

Disclosure of Interest: None declared.

PO670-TUE

Modification of ABO(H) blood group antigens on human vwf with plasma glycosyltransferase

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Background: The plasma von Willebrand factor (VWF) is known to have ABO(H) blood group antigens consistent with those in red blood cells. Since the plasma concentration of VWF is reported to vary according to blood groups, its glycosylation is possible to concern the

onset of von Willebrand disease, or may further affect its treatment. VWF glycosylation mechanism should be important but not yet clarified. Plasma contains glycosyltransferase that constructs ABO(H) blood group antigens.

Aims: We have investigated whether H-type glycan (blood group O) of VWF is possible to elongate and is changeable into A- or B-types by plasma or recombinant glycosyltransferase.

Methods: (i) Bovine serum albumin conjugated with H-type glycan (H-BSA) or (ii) VWF prepared from O-type plasma (O-VWF) was incubated with either A-type plasma (AP) or recombinant A-glycosyltransferase (rATase), in the presence or absence of donor sugar (UDP-GalNAc) using a GALSERVE AB kit. Blood group A antigens were detected using the enzyme-linked immunosorbent assay (ELISA) or the western-blot analysis.

Results: The H-BSA or O-VWF were successfully glycosylated and transformed into A-type after incubation with higher concentration of rATase only in the presence of UDP-GalNAc. In the case of AP used, blood group A antigen was attached on H-BSA but not on O-VWF after incubation with AP in the presence of UDP-GalNAc.

Conclusion: These results suggest that plasma glycosyltransferase might have little effect on the construction of blood group antigens of VWF. Considering the very low concentration of substrates in plasma, VWF might be glycosylated within the endothelial cells before secretion into plasma.

Disclosure of Interest: None declared.

PO671-TUE

Reference value of von Willebrand factor activity by immuno-turbidimetry method

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Background: von Willebrand factor (vWF) is a multimeric glycoprotein that function as a bridge between platelets and subendothelial tissue, and also acts as a carrier of factor VIII and protect from degradation. Hereditary deficiency of vWF or von Willebrand disease (vWd) can be classified as type I, II, and III. Type I and type III of vWd are characterized by partial and total quantitative defect, respectively, while type II vWd is a qualitative defect characterized by normal level but decreased vWF activities. Therefore determination of vWF activities is preferred than vWF level since it can detect qualitative defect of vWF. It has been known that vWF concentration is affected by ABO blood group.

Aims: The aim of this study is to determine reference value of vWF activities in O blood group and non O blood group of Indonesian peoples.

Methods: One hundred twenty healthy subjects aged 22 – 62 year-old, comprised of 60 with O blood group O and 60 with non-O blood group were enrolled for this study. The activities of vWF were determined by immuno-turbidimetric method using INNOVANCE[®] vWF Ac from Siemens Healthcare Diagnostic, Germany and automated coagulometer CS-2100i from Sysmex Corp, Japan. Principle of measurement was increased turbidity due to agglutination after the addition of particle coated with anti GPIb and recombinant GPIb (two gain-function mutations) to plasma sample containing vWF.

Results: The result of vWF activities in O blood group was $74.8\% \pm 25.3\%$ ($X \pm SD$) while in non-O blood group was $101.9\% \pm 32.1\%$ ($X \pm SD$). There was a significant difference between vWF activities among two groups ($P = 0.000$).

Conclusion: The reference value of vWF activities in O blood group was 24.2% – 125.4% while in non-O blood group was 37.7% – 166.1%. The vWF activities of O blood group was significantly lower than non-O blood group.

Disclosure of Interest: R. Dharma Grant/Research Support from: PT Sysmex Indonesia, Y. Mimanda: None declared.

PO672-TUE

Analysis of plasma von Willebrand factor and complement factor H polymorphisms in patients with age-related macular degeneration

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Background: Age-related macular degeneration (AMD) is the leading cause of severe visual dysfunction in people aged over 50 years in industrialized countries. *Complement factor H (CFH)* gene has been demonstrated to be the major AMD susceptibility gene. Among the various single nucleotide polymorphisms (SNP) in the *CFH* gene, Y402H and V62I have been most intensively evaluated. Recently, the interaction between CFH and von Willebrand factor (VWF) has been reported.

Aims: To evaluate the pathophysiology of AMD, we investigated the association between plasma VWF and the *CFH* polymorphisms Y402H and V62I in patients with AMD.

Methods: Seventy-three patients (53 male, 21 female) with treatment-naïve exudative AMD were included. As controls, 96 patients with cataract without AMD (age and sex matched) were also included. We analyzed plasma VWF antigen (VWF:Ag), VWF multimers, and the genotype for the *CFH* p.Y402H (c.1204 T>C) and p.V62I (c.184 G>A) using restriction fragment length polymorphism.

Results: As for Y402H, wild type (TT) was detected in 66 patients and heterozygote (TC) in 8 patients. The frequency of Y402H in patients with AMD showed no significant difference compared with those in controls. In V62I mutation, wild type (GG) was found in 46 patients, heterozygote (GA) in 26, homozygote (AA) in 2. The incident rate of AMD in wild type of V62I was significant higher than those in another type. Plasma levels of VWF:Ag of patients with AMD ($175 \pm 82\%$) were significantly higher than those of controls ($138 \pm 46\%$). However, we did not detect relation between VWF:Ag and the *CFH* Y402H or V62I. In addition, we found three male patients with severe AMD complicated with vitreous hemorrhage. Plasma VWF:Ag levels of these patients were very high. Unusually-large VWF multimers were detected in all 3 patients with severe AMD.

Conclusion: We found the relation between the risk of AMD onset and SNP V62I. Because plasma VWF:Ag were significantly increased in AMD patients, AMD might be developed in association with thrombosis in eye.

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PO673-TUE

Improved resolution of high molecular weight multimers of recombinant von Willebrand factor–albumin fusion product by agarose electrophoresis/western blotting

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Background: von Willebrand disease occurs as a result of structural or functional defects to von Willebrand factor (VWF), a critical mediator in platelet aggregation and adhesion. A recombinant VWF-albumin fusion protein (rVWF-FP) is currently under development with the aim of increasing the plasma half-life for prophylactic administration.

VWF and rVWF-FP are both large proteins capable of forming disulfide-linked multimers over a wide distribution of molecular weights (MWs), with high MW (HMW, 5000–10,000 kDa) and ultra-large (UL, > 10,000 kDa) multimers being the most effective mediators of platelet aggregation. However, consistent visualisation of HMW and UL multimers by immunoblotting has proved challenging, possibly due to their large size impeding transfer during electroblotting.

Aims: To address issues in the VWF multimer assay to improve the detection and visualisation of HMW and UL VWF and rVWF-FP species

Methods: A dithiothreitol wash step to reduce disulfide bonds prior to electroblotting was incorporated into the standard agarose-based electrophoresis/immunoblotting procedure for VWF multimer distribution evaluation. Additionally, the use of fluorescence-based antibody detection was evaluated in place of traditional chromogenic-based detection

Results: The inclusion of the dithiothreitol reduction step was found to improve transfer of HMW multimers of both rVWF-FP and VWF to the polyvinylidene fluoride membrane. Furthermore, the use of fluorescent moiety-conjugated antibodies resulted in superior resolution of HMW multimers compared with traditional enzyme-conjugated immunologic detection. Finally, a close positive correlation between relative abundance of HMW multimers and platelet-binding capacity was demonstrated.

Conclusion: Optimisation of the analytical procedure for the VWF multimer assay provides improved resolution and detection of HMW multimers. It is hoped that these assay improvements will facilitate future development of VWF products.

Disclosure of Interest: J. Hey Shareholder of: CSL Limited, Employee of: CSL Limited, T. Barnes Employee of: CSL Limited, M. Zimmermann Employee of: CSL Limited, B. Yarwood Employee of: CSL Limited, C. Pical Employee of: CSL Limited, H. Lind Employee of: CSL Behring

PO674-TUE

Analysis of oxaliplatin-based chemotherapy induced liver injury in patients with advanced colorectal cancer with special references to von Willebrand factor

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Background: Oxaliplatin-based chemotherapy has been widely used for treatment of advanced colorectal cancer (CRC). Sinusoidal obstruction syndrome (SOS) due to oxaliplatin is one of the serious chemotherapy-associated liver injury (CALI) in patients with CRC. We previously reported that unusually-large von Willebrand factor multimers (UL-VWFM) were observed in SOS patients after stem cell transplantation.

Aims: To investigate the pathophysiology of CALI after oxaliplatin-based chemotherapy.

Methods: Twenty-three patients with advanced CRC who received oxaliplatin-based chemotherapy with ($n = 6$) and without ($n = 17$) bevacizumab (Bev) were included. Of these, 10 patients were diagnosed with CALI. We analyzed VWF antigen (VWF:Ag) and VWFM before and until 5 months after chemotherapy.

Results: Splenomegaly was found only in patients who were treated without Bev. Plasma levels of VWF:Ag and serum levels of aspartate transaminase (AST) were increased with the number of chemotherapy in patients with splenomegaly, but not without. We compared between the oxaliplatin-based chemotherapy with and without ($n = 17$) Bev group. Both plasma VWF:Ag and serum AST were increased as the number of chemotherapy in patients without Bev, but not with.

VWFM analysis in patients without bevacizumab showed both the presence of UL-VWFM and the absence of high molecular weight VWFM (H-VWFM) during the chemotherapy in the same patient. In contrast, no apparent changes in VWFM pattern were observed in patients with Bev. Histological findings of liver in patient without Bev demonstrated sinusoidal dilatation and microthrombi in the sinusoids, many of which were positive for both anti-IIb/IIIa antibody and anti-VWF antibody.

Conclusion: Plasma UL-VWFM might be increased by the damage of endothelial cells due to oxaliplatin-based chemotherapy and induced platelet thrombi in sinusoids. H-VWFM and platelets were consumed in platelet thrombi, which caused CALI and splenomegaly. Bev might prevent CALI and splenomegaly through inhibiting platelet thrombi.

Disclosure of Interest: None declared.

PO675-TUE

Desmopressin treatment improves platelet function under flow in patients with postoperative bleeding

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Background: Patients undergoing major cardiothoracic surgery are subjected to dilution due to transfusion of fluids and blood components. These patients may experience perioperative bleeding and are frequently treated with the endothelial-activating agent desmopressin.

Aims: We investigated the effect of desmopressin administration on VWF-dependent coagulant and platelet functions.

Methods: Patients ($n = 13$) who had undergone cardiothoracic surgery with postoperative bleeding were included after ethical approval and informed consent. Blood was taken immediately before and 1 h after desmopressin ($0.3 \mu\text{g kg}^{-1}$ body weight) administration. Coagulant function was assessed by aPTT, prothrombin and factor VIII levels, thrombin generation and thromboelastometry. von Willebrand factor (VWF) antigen levels were determined by nephelometry, multimer analysis by horizontal discontinuous gel electrophoresis. Thrombus formation was studied by whole blood perfusion over a collagen surface (shear rate of 1000 s^{-1}).

Results: Desmopressin treatment did not change thrombin generation nor thromboelasticity. Also coagulation factor levels (except factor VIII) and coagulation times were unchanged. On the other hand, desmopressin raised the already high plasma levels of VWF from 124 (104–159) to 156 (125–185) IU mL^{-1} ($P < 0.05$), due to accumulation of high-molecular-weight VWF multimers. Increased collagen-dependent thrombus formation was detected after treatment, which was accompanied by a higher fraction of procoagulant, phosphatidylserine-exposing platelets. Markers of thrombus formation correlated with the plasma levels of VWF. Control experiments confirmed a major contribution of VWF to thrombus formation and procoagulant activity.

Conclusion: Desmopressin treatment of patients with bleeding complications induces the release of high-molecular-weight VWF multimers, which enhance platelet activation and thrombus formation under flow conditions. This may improve haemostasis in these patients.

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tapharma Biopharmaceuticals, J. Heemskerk: None declared, P. Van Der Meijden: None declared.

PO676-TUE

O to avoid myocardial infarction

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Background: Blood group is a risk marker for myocardial infarction; it is also a risk marker for bleeding, it is not clear why. The ABO antigens are expressed on both platelet glycoproteins and von Willebrand Factor (VWF). VWF captures rolling platelets from flowing blood at sites of vascular injury. Our hypothesis is that expression of AB determinants on platelet surface glycoproteins including GpIb may modulate interaction with VWF.

Aims: To test our hypothesis we perfused blood from healthy normal donors over a surface of VWF purified from pooled human plasma at rates of arterial shear (1500 s^{-1}).

Methods: Microlitre quantities of whole blood were fluorescently labelled and perfused through a parallel perfusion flow chamber. 1000 images were recorded in real time at a frame rate of 30 frames per second. Using a validated novel tracking algorithm we measured the number of platelets translocating across VWF, the distance travelled and velocity of movement for each platelet and the number of platelets stably adhered to the surface.

Results: Blood from 60 volunteers was analyzed. Platelets from volunteers with blood group O ($n = 20$) travelled a distance of $7.9 \pm 2.5 \text{ mM}$ (mean $\pm$ SD) across the VWF surface compared to $6.7 \pm 2.9 \text{ mM}$ for non-O individuals ($n = 40$ $P = 0.009$). In Type O individuals there was a greater number of translocating platelets than static platelets compared to non-O donors (ratio 2.6 ± 1.0 vs. 2.0 ± 1.1 , $P = 0.006$).

Conclusion: The results of this investigation demonstrate that platelets from type O donors interact less with plasma-derived human VWF. These results are independent of donor VWF plasma concentrations. Group O platelets expressing terminal H antigen determinants travel further across and stick less to VWF. Collectively, these data suggest a novel mechanism through which specific ABO blood groups may modulate risks for both thrombosis and bleeding respectively.

Disclosure of Interest: None declared.

PO677-TUE

von Willebrand factor's shear-and-time dependent degradation under pulsatile shear through a capillary shear system

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Background: von Willebrand Factor (VWF) is a pivotal protein in coagulation under shear conditions. The VWF activity is correlated to the size of the multimers which are degraded by ADAMTS13. However, excessive degradation may be caused by medical devices, such as ventricular assist devices, which can lead to an acquired von Willebrand Disease. The extent of degradation is correlated to both the shear stress and exposure time to such forces, but their relative importance in VWF degradation is not known, especially in such medical devices. In order to better understand the relationship between shear stress and exposure time in the process of VWF degradation we inves-

tigated the effect of such conditions under pulsatile conditions at high shear stresses (500–1500 dynes cm^{-2}) and short exposure times (ms).

Aims: Quantitatively investigate the VWF degradation in a capillary system under pulsatile shear to determine the relative importance of shear stress and exposure time.

Methods: Pooled platelet poor plasma (PPP) was prepared from 5 volunteers with IRB-approved consents. PPP was exposed repeatedly (up to 300 $\times$) to shear pulses at 37 °C when pumped through tubing of 0.38 mm inner diameter at preselected flowrates. The wall shear stresses ranged 500–1500 dynes cm^{-2} with exposure times inversely ranging from 130 to 40 ms for each pulse. VWF in the sheared PPP was analyzed using electrophoresis in 2% agarose gel followed by Western Blot and then quantified by densitometry.

Results: Under pulsatile shear exposures with increasing shear stress (500–1500 dynes cm^{-2}) and proportionally decreasing time from (130–40 ms), the high and intermediate molecular weight VWF degradation increased gradually from 36% to 76% ($P = 0.004$, $n = 3$) compared to the controls. The effect of shear stresses on VWF degradation is 1.71 ± 0.06 times of the exposure times.

Conclusion: The results indicate that shear stress effects outweigh the shear exposure times with respect to VWF degradation under pulsatile shear exposures in this capillary system.

Disclosure of Interest: None declared.

PO678-TUE

Longitudinal analysis of von Willebrand factor (VWF) levels in the phase 3 a-long study of recombinant factor VIII FC fusion protein (RFVIII FC) in subjects with hemophilia A demonstrates different magnitudes of temporal variability

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Background: Limited data which assess longitudinal von Willebrand factor (VWF) levels in subjects with severe hemophilia A exist in the literature.

Aims: To assess VWF antigen (VWF:Ag) level stability and factors that may affect these levels using data from a large, longitudinal study of severe hemophilia A.

Methods: VWF:Ag levels were measured periodically during A-LONG, a phase 3 study of rFVIII FC in males ≥ 12 years with severe hemophilia A ($< 1 \text{ IU dL}^{-1}$ endogenous FVIII). Analyses were conducted to assess the relationship between potential covariates (eg, age, weight, blood type, study arm, pre-study treatment, HCV, HIV, geographic location, bleeding, number of target joints, serum IgG concentration) and (i) baseline VWF:Ag (multiple regression model) and (ii) change in VWF:Ag over time (slope; mixed effects model). Characteristics distinguishing subjects with and without large changes in VWF:Ag (absolute change $\geq 30\%$) were also assessed (logistic regression model).

Results: At screening, VWF:Ag was assessed in 158 subjects; it was higher in older subjects and with non-O blood type (both $P < 0.05$). Baseline VWF levels were positively correlated with rFVIII FC half-life ($r = 0.67$; $P < 0.001$). Over the study period, VWF:Ag was generally stable (estimated slope, 0). For the time period from screening to Day 0 (~7 wk), subjects ($n = 155$) were categorized based on change in VWF:Ag as stable ($\pm < 15\%$; $n = 87$), moderately stable ($\pm \geq 15\%$ to $< 30\%$; $n = 41$), or fluctuating ($\pm \geq 30\%$ [$\geq 30\%$: median, 38%; $\geq 30\%$: median, -44%]; $n = 27$); no covariates were identified in this study to be statistically significantly associated with changes in VWF:Ag.

Conclusion: VWF:Ag was generally stable during A-LONG, and higher in older subjects and with non-O blood type; 17% of subjects

showed a temporary fluctuation in VWF:Ag. Notably, covariates of VWF levels may differ in this selected group vs. a general population. Additional research is needed to identify other potential covariates associated with changing VWF:Ag levels.

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PO679-TUE

Evaluation of age and glycan-related changes on von Willebrand factor (VWF) clearance in a VWF deficient mouse model

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Background: Studies have suggested that differences in von Willebrand factor (VWF) glycosylation related to ABO blood type and cellular origin can influence its rate of clearance. Previous results obtained with surrogate markers of VWF clearance showed that a reduced clearance contributes to the increase in VWF levels with age. This could be the result of changes in VWF clearance receptors with age, or intrinsic changes to VWF that affect its clearance.

Aims: To evaluate age- and glycan related changes on VWF clearance in VWF^{-/-} mice.

Methods: Young (9-weeks) and/or old (> 78 -weeks) C57BL/6 VWF^{-/-} mice were injected via tail vein with VWF (200 U kg^{-1}) and samples collected via retro-orbital bleeding. Recombinant mouse (r-mVWF) and human VWF (r-hVWF) were produced in HEK293 cells. Plasma-derived mouse VWF (pd-mVWF) was produced in murine hepatocytes via hydrodynamic injections. Plasma-derived human VWF (pd-hVWF) was either commercially available or obtained from young and old blood type O (23 ± 2 years/70 ± 6 years) and non-O donors (23 ± 1 years/74 ± 7 years). Half-lives [95% CI] were calculated by a one-phase exponential decay model. Best-fit values are shown where no difference was observed.

Results: No difference was found in VWF clearance between young and old mice for r-mVWF (36 [33–41] min, $P = 0.78$), pd-mVWF (392 [344–458] min, $P = 0.12$) or pd-hVWF (56 [49–65] min, $P = 0.53$), suggesting that no significant changes in VWF clearance receptors occur with aging. Age and ABO differences in VWF molecules were also evaluated, but no difference was found between young and old pd-hVWF (62 [55–72] min, $P = 0.98$), independent of blood type (63 [56–71] min, $P = 0.11$). The half-life of r-hVWF was not different to r-mVWF ($P = 0.28$), but they were significantly shorter than the corresponding pd-VWF products (m: 37 vs. 346 min, $P < 0.0001 \text{ h}^{-1}$: 29 vs. 55 min, $P < 0.006$).

Conclusion: The VWF^{-/-} mouse model is able to demonstrate differential clearance of VWF derived from different cellular sources, but shows limited recognition of ABO differences or changes in VWF with age.

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PO680-TUE

Annexin A2 SNP regulates plasma von Willebrand factor levels and is enriched in patients with von Willebrand disease

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Background: von Willebrand Disease (VWD) type 1 is a heterogeneous bleeding disorder. In only 70% of VWD type 1 cases the cause is related to mutations in the von Willebrand Factor (VWF) gene. vWF is stored in Weibel Palade Bodies (WPB) present in endothelial cells. Exocytosis of WPB is regulated by the phospholipid binding protein Annexin A2 (AnxA2).

Aims: We studied whether a common *AnxA2* SNP (c. 346G>T, rs17845226) in the *AnxA2* gene, which causes a Valine to Leucine amino acid change, may affect this process by measuring the VWF levels in controls and by determination of its prevalence in patients with von Willebrand Disease (VWD).

Methods: Plasma and DNA was collected from a control group ($n = 80$) and presumed VWD patients ($n = 94$). rs17845226 was genotyped by Sanger sequencing. VWF Antigen (VWF:Ag), VWF Ristocetin cofactor activity (VWF:RCO) and vWF collagen binding activity levels (VWF:CB) were measured in controls, and stratified by heterozygous carriers and non carriers (WT). The SNP data of a genome-wide association study (GWAS) control population ($n = 1502$) of the Nijmegen Biomedical Study (NBS) was used to calculate the Odds ratio (OR) with its 95% confidence interval in patients with VWD.

Results: Significant lower levels of VWF:CB were determined in heterozygous carriers of the SNP as compared to WT controls (92 vs. 114 IU dL⁻¹, $P = 0.029$). Both VWF:Ag (88 vs. 107 IU dL⁻¹, $P = 0.096$) and VWF:RCO levels (88 vs. 107 IU dL⁻¹, $P = 0.096$) showed a trend to lower levels. The prevalence of the c.346T allele was shown to be increased in patients with VWD, more specifically in type 1 (OR=3.03, 1.25–7.08) and 2N (OR=2.88, 1.30–6.15).

Conclusion: The c. 346T allele in the *AnxA2* gene causes lower VWF antigen and activity levels in healthy controls independent of blood group. Furthermore, patients with VWD carry more often the c. 346T allele. The significantly higher odds ratio in type 2N is partly due to a compound heterozygous effect on Nijmegen type VWD/2N.

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Acquired coagulation disorders/ inhibitors

PO001-WED

Acquired haemostatic disorders by orthotopic liver transplantation

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Background: Transmission of hemostatic disorders after orthotopic liver transplantation (OLT) is rare. FXI is produced by the liver and

its deficiency may cause excessive blood loss. Ashkenazi/Iraqi ancestry Jews and Israeli Arabs are affected. FV is mainly synthesized by the liver. A single point mutation in the FV gene at one of the cleavage sites for activated protein C (APC) is responsible for the coagulation defect (FV:Q506). This results in a properly activated FV, which is inactivated in a reduced rate by APC (APC resistance, APCR).

Aims: To report on two patients who underwent OLT and acquired hemostatic disorders.

Methods: APCR was done by using Factor V Leiden (APCTM Resistance V) reagents and FVIII, FIX, FXI activity was detected by using a specific factor deficient plasma. All of the above tests were done using Instrumentation Laboratory (MA, USA) reagents and their ACL TOP system. FV gene mutation was done by real time PCR using fluorescence resonance energy transfer probe (FRET) (Thermo Scientific MA, USA).

Results: One patient was a 61 years old man of Libyan Jewish ancestry, referred before lung biopsy because of prolonged PTT (73.6 s, normal < 40 s) 2 years after OLT for cirrhosis and hepatic carcinoma. Antiphospholipid antibodies were not detected, FVIII 208%, FIX 121%, FXI 4%(normal > 50%) with no specific antibodies. The second patient was a 48 years old woman with right jugular and subclavian deep vein thrombosis 2 months after OLT because of fulminate hepatic failure. Screening for thrombophilia revealed low APCR (1.49, $n > 2$) with no detected mutation in the F V gene.

Conclusion: Transmitted hemostatic disorders after OLT depend on the genotypes of the recipient and the donor livers and may cause severe/life threatening complications. Being aware of the potential transmission of inherited coagulation defects and the availability of simple and affordable assays, aid in their early detection and management in the transplant donor and recipient.

Disclosure of Interest: None declared.

PO002-WED

Correlation between activated protein C (APC) resistance and STNFR2 in alzheimer disease patients carrying 'EPSILON'4 allele of the apolipoprotein E

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Background: Presence of the $\epsilon 4$ allele of the apolipoprotein E (APOE) gene is the main genetic risk factor for developing Alzheimer disease (AD), whose allele leads to production of an APOE isoform unable to promote proper amyloid- β -peptide (A β) metabolism, favoring its deposition in neurons. This seems to be a key event in the pathogenesis and progression of AD, in addition to inflammatory and hemostatic mechanisms reported by several studies as important factors exacerbating the neurodegenerative process. Unpublished data of our research group has shown a significantly higher number of AD patients with APC resistance, compared to the control group, which indicates a hypercoagulable state. Plasma levels of soluble receptors for TNF- α (sTNFR1 and sTNFR2) have been reported as inflammatory activity markers in patients with AD and mild cognitive impairment.

Aims: To investigate whether there is correlation between activated protein C (APC) resistance and inflammatory markers in $\epsilon 4$ allele carriers vs. noncarriers AD patients.

Methods: We evaluated 35 Brazilian AD patients $\epsilon 4$ allele carriers and 30 noncarriers patients. APC resistance was determined by thrombin generation assay, method of CAT (Calibrated Automated Thrombogram), performed in the presence and absence of APC, followed by calculation of nAPCsr index (normalized activated protein C sensitivity ratio). Plasma levels of soluble receptors for TNF- α (sTNFR1 and sTNFR2) were measured by ELISA method. Polymorphisms of the APOE gene were identified by PCR-RFLP.

Results: Only patients with AD carrying $\epsilon 4$ allele showed a strong correlation between nAPCsr and sTNFR2 plasma levels ($P < 0.001$; $r = 0.808$ by Spearman test).

Conclusion: These results suggest that APC resistance indicating hypercoagulability in AD patients appears to be related to the inflammatory status and exacerbated by the presence of $\epsilon 4$ allele, confirming its unfavorable character.

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PO003-WED

Persistent international normalized ratio (INR) elevation associated with olmesartan-induced enteropathy in a patient receiving warfarin

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Background: Warfarin's depletion of Vitamin (Vit) K stores may be reversed by increasing Vit K intake. Oral Vit K is safe, and predictably lowers INR within 24–48 h in non-urgent situations. Vit K malabsorption can lead to increased warfarin effect and higher INR. Inflammatory bowel conditions like celiac or Crohn disease can cause malabsorption. There have also been reports of angiotensin II receptor blocker induced sprue-like enteropathy.

Aims: To report a case of persistently elevated INR despite high dose oral Vit K in a patient receiving warfarin who developed colitis associated with intermittent olmesartan use.

Methods: A 65-year-old female of West Indian descent with inflammatory colitis and on warfarin for deep vein thromboses, atorvastatin, and olmesartan. The patient was evaluated for persistently elevated INR > 9.0 , 3 months after initiation of warfarin. The patient had mild diarrhea and weight loss after re-introduction of olmesartan and recent intestinal biopsy showed extensive villous atrophy consistent with autoimmune colitis. The patient had no signs of bleeding. The coagulopathy was resistant to reversal despite with-holding warfarin for 13 days and 25 mg of oral Vit K. Previous elevation of INR normalized over 48 h with 5 mg of oral Vit K. Celiac (TTG) screening was negative. CBC and liver panel were normal. Haemostasis studies revealed deficiency in Vit K-dependent clotting factors. Vit K malabsorption due to olmesartan-associated enteritis was suspected.

Results: After further administration of oral Vit K 10 mg daily for 3 days, INR returned to 1.1. Clinically, colitis symptoms resolved with olmesartan discontinuation and initiation of prednisone with subsequent intestinal biopsy showing improved duodenal appearance.

Conclusion: This case underscores the importance of investigating severe warfarin-associated coagulopathy including drug-induced malabsorption. When malabsorption is identified, oral or intravenous Vit K may be required in doses greater than expected to normalize INR.

Disclosure of Interest: None declared.

PO004-WED

Differentiation between pro- and anticoagulant activity demonstrates rebalanced thrombin generation in liver cirrhosis

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Background: Almost all pro- and anticoagulant factor levels are reduced in liver cirrhosis patients. However, cirrhosis patients show no clear bleeding or thrombotic phenotype and thrombin generation (TG) is (almost) unaffected.

Aims: We aim to determine changes in the underlying processes of TG in liver cirrhosis and how this affects TG.

Methods: We have recently developed a method to split a TG curve into its two underlying processes: prothrombin conversion and thrombin inactivation. We applied this technique to a set of 25 healthy subject and 25 liver cirrhosis patient samples.

Results: Prothrombin levels were decreased in patients ($P < 0.001$). The plasma level of antithrombin (AT) was decreased in cirrhosis patients ($P < 0.001$), whereas the α_2 Macroglobulin level (α_2 M) was significantly increased ($P < 0.001$). Both prothrombin conversion and thrombin inactivation are markedly reduced in cirrhosis patients. The total amount of prothrombin converted during TG was reduced in patients ($P < 0.001$), but the maximum rate of prothrombin conversion (i.e. the activity of the prothrombinase complex) was unchanged. The inactivation of thrombin by AT was reduced in patients ($P < 0.001$), and thrombin inactivation by α_2 M was increased ($P < 0.001$). The overall result was a reduction of thrombin decay capacity in cirrhosis patients ($P < 0.001$). *In silico* normalization of prothrombin conversion in cirrhosis patients caused the ETP to rise to thrombotic levels (2878 ± 1323 nM·min) and normalization of AT levels caused a pronounced reduction of peak height and ETP ($P < 0.001$).

Conclusion: In conclusion, despite large differences in prothrombin conversion and thrombin inactivation, TG in liver cirrhosis patients remains within the normal range (rebalanced), and normalization of either process would induce a thrombotic or bleeding phenotype.

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PO005-WED

Hemostatic effects of regenerative therapy in patients with alcoholic cirrhosis-a developing country experience

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Background: Granulocyte-colony stimulating factor (G-CSF) is the most commonly used growth factor for mobilizing bone marrow stem cells which are postulated to carry the potential to repopulate the liver parenchyma and allow maturation of its progenitors into hepatocytes. While patients with cirrhosis suffer from profound hemostatic defects leading to hemorrhagic as well as prothrombotic derangements, it is hypothesized that regenerative therapies like G-CSF might lead to improvement of these coagulopathic anomalies.

Aims: To detect coagulation profile improvement in alcoholic cirrhotics, post G-CSF therapy using viscoelastic and conventional coagulation techniques.

Methods: A prospective study, approved by the Institutional Ethics Committee (comprising 45 patients of biopsy proven compensated

alcoholic cirrhosis with prior informed consent) was undertaken. Blood samples were taken prior to and post 60 day G-CSF therapy ($5 \mu\text{g kg}^{-1}$). The post therapy samples were collected from only 39 patients (6 dropouts due to poor compliance in 4, adverse drug reaction in 2). The pre(PR) and post(PO) therapy prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time, proteinC, antithrombin, factor VIII, von Willebrand factor (coagulometric assays), platelet count (by cell counter) and activated clotting time (ACT), clot rate and platelet function (viscoelastic method, sonoclot) were analysed using paired t test. *P* value considered significant if < 0.05 .

Results: Mean age was 49.1 ± 8.5 years, 93% of which were males. PO samples showed significant improvement ($P < 0.05$) in Factor VIII levels (mean $160.1 \pm 97.6 \text{ IU dL}^{-1}$) as compared to PR samples (mean $224.98 \pm 90.8 \text{ IU dL}^{-1}$). While the previously prolonged PT, INR and ACT values improved, the difference was not significant. No change was seen in other parameters.

Conclusion: To conclude, the 60 day G-CSF therapy led to normalization of factor VIII levels (vis-a-vis other coagulation parameters) in alcoholic cirrhotics. Larger studies are needed to validate the results.

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PO006-WED

Two Japanese cases of acquired factor V inhibitor which were initially diagnosed with congenital factor V deficiency

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Background: Acquired factor V (FV) inhibitor is a rare bleeding disorder, and the clinical phenotypes range from asymptomatic laboratory abnormalities to fatal bleeding episodes. A diagnosis of acquired FV inhibitors is based on the presence of inhibitors against FV by mixing studies and the Bethesda method.

Aims: We report here two Japanese cases of acquired FV inhibitor that were initially diagnosed with congenital FV deficiency by mixing studies.

Methods: We retrospectively reviewed patients diagnosed with acquired FV inhibitor in our hospital.

Results: Case 1: A 55-year-old woman with hematuria was referred to our hospital. Coagulation tests revealed marked prolongation of aPTT (103 s) and PT (> 60 s). Her FV activity was 3.0% and mixing studies of patient and normal plasma could correct aPTT, so she was diagnosed with congenital FV deficiency. A year later she developed intramuscular bleeding of her left thigh and was admitted to our hospital. Mixing studies failed to correct, which suggested the presence of inhibitors. As we considered the possibility of acquired FV inhibitor, prednisolone (PSL) was initiated, and then coagulation tests almost normalized several days later. Case 2: An 84-year-old woman with urinary tract infection was admitted to neighboring hospital. Coagulation tests revealed prolongation of aPTT and PT (aPTT 78 s, PT-INR 3.74). Her FV activity was 15.9%, and mixing studies could correct aPTT, so she was diagnosed with congenital FV deficiency. A month later, she was admitted to the hospital because of intra-articular bleeding of her left knee. At this point, she was diagnosed with acquired FV inhibitor because mixing study failed to correct. She was administered PSL, then coagulation studies improved and FV inhibitor disappeared.

Conclusion: We reported two Japanese cases of acquired FV inhibitor, which were initially diagnosed with congenital FV deficiency. In

patients with acquired FV inhibitor, mixing studies at the early phase of the disease may result in a misleading diagnosis.

Disclosure of Interest: M. Bingo Consultant for: Bayer, Novo Nordisk, Paid Instructor at: Bayer, Baxter, Speaker Bureau of: Bayer, Baxter, K. Shinozawa Grant/Research Support from: Baxter, Paid Instructor at: Bayer, Novo Nordisk, Speaker Bureau of: Baxter, Bayer, Pfizer, Novo Nordisk, T. Hagiwara Consultant for: Baxter, Novo Nordisk, CSL Behring, Biogen Idec, Paid Instructor at: Bayer, Speaker Bureau of: Baxter, Pfizer, H. Inaba Grant/Research Support from: Baxter, Consultant for: Baxter, Paid Instructor at: Bayer, Speaker Bureau of: Baxter, Bayer, Pfizer, T. Suzuki Grant/Research Support from: Novo Nordisk, Consultant for: Pfizer, Novo Nordisk, Baxter, Paid Instructor at: Bayer, Novo Nordisk, Speaker Bureau of: Baxter, Bayer, Novo Nordisk, Sekisui Medical, K. Amano Consultant for: Baxter, Bayer, Novo Nordisk, Paid Instructor at: Baxter, Bayer, Speaker Bureau of: Baxter, Pfizer, Biogen Idec, Bayer, Kaketsuken, Novo Nordisk, K. Fukutake Grant/Research Support from: Baxter, Bayer, Pfizer, Kaketsuken, Japan Blood Products Organization, CSL Behring, Novo Nordisk, Consultant for: Baxter, Pfizer, Biogen Idec, Bayer, CSL Behring, Kaketsuken, SRL, LSI Medience, Novo Nordisk, Paid Instructor at: Baxter, Bayer, Speaker Bureau of: Baxter, Bayer, Pfizer, Biogen Idec, Novo Nordisk, CSL Behring, Roche Diagnostics, Fujirebio Inc, Sekisui Medical.

PO007-WED

Treatment of acquired factor V inhibitors resulting in disseminated intravascular coagulation

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Background: Factor V (FV) inhibitors are a very rare acquired coagulopathy.

Aims: To raise awareness of complications in treating FV inhibitors.

Methods: We report 2 cases presenting within a year, in a regional UK haemophilia service covering a population of 2,600,000.

Results:

Case 1: A 43 year old female had an uncomplicated apronectomy with liposuction 2 weeks prior to presentation with a retroperitoneal bleed. Screening tests showed PT 65 s and APTT 180 s (no correction on 50:50 mixing with normal plasma). There was an isolated reduction of FV 0.03 IU mL^{-1} and a Bethesda assay set up against FV demonstrated an inhibitor. She received FEIBA, platelets, intravenous immunoglobulin (IVIg) and prednisolone. 7 days later she deteriorated with possible sub-arachnoid bleeding. Despite recombinant Factor VIIa, she died. Prior to death her fibrinogen level was 0.5 g L^{-1} . Post mortem showed intracranial bleeding with microvascular thrombosis.

Case 2: A 61 year old female presented with intra cranial bleeding. Full blood count was normal and PT was 25 s, APTT 73 s (50:50 mix, no correction), Clauss fibrinogen 4.6 g L^{-1} . She had an isolated reduction in FV to 0.06 IU mL^{-1} with a FV inhibitor of 6 BU. There was a history of a severe adverse reaction to Naproxen approximately 4 weeks prior. Platelet transfusion, FEIBA ($50 \text{ IU kg}^{-1} \text{ bd}$) and prednisolone were started. After 3 days she developed haematuria and laboratory evidence of disseminated intravascular coagulation (DIC). FEIBA was stopped; fibrinogen concentrate, platelet transfusions, as a source of FV, and IVIg were given. The FV level rose rapidly and remains above 1 IU mL^{-1} whilst steroids are reducing.

Conclusion: FV inhibitors have been associated with topical bovine thrombin during surgery, autoimmunity, malignancy and drugs, especially antibiotics. In our cases, medication was possibly the trigger. Treatment with FEIBA was associated with DIC in both of our cases.

We propose that platelets should be considered as first line therapy in FV inhibitor patients.

Disclosure of Interest: None declared.

PO008-WED

Lupus anticoagulant – hypoprothrombinemia syndrome in a 7-year old girl: a case report

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Background: Lupus anticoagulant (LA) can be associated with bleeding as a consequence of hypoprothrombinemia.

Aims: We present the report of a patient with hemorrhagic symptoms in combination of hypoprothrombinemia and LA.

Methods: A 7 year-old girl was admitted with epistaxis for 5 days. On physical examination, she had scleral icterus, echimosis and hepatomegaly. The complete blood count was normal except hemoglobin of 47 g L^{-1} . The coagulation screening showed a prolonged prothrombin time (PT) (60.8 s) and activated partial thromboplastin time (aPTT) (118.6 s). Although a packed fresh frozen plasma was administered to the patient, the prolonge of PT and aPTT continued. Therefore, the mixing study was performed but both PT and aPTT did not improve.

Results: Factor assay revealed a low prothrombin level (4.46 %). The lupus anticoagulant and anti nuclear antibody were found positivity but anti DS-DNA was negativity in advanced laboratory examination of patient respectively. Further evaluation revealed the presence of immunoglobulin (Ig) G an M anticardiolipin antibodies, IgG and IgM anti- β -2-glycoprotein antibodies, IgG and IgM anti phosphatidylserin antibodies. After 5 days of mega dose methylprednisolone ($30 \text{ mg kg}^{-1} \text{ day}^{-1}$) was administered to the patient, PT (13.6 s) and a PTT (38.4 s) were measured normal and directly Coombs test was found negatively. Therefore, mega dose methylprednisolone treatment was stopped. One month after stopping of the treatment with mega dose methylprednisolone she had again both prolonged PT and aPTT, and the positivity of directly Coombs test. In this period, it was revealed the presence of immunoglobulin (Ig) G an M anticardiolipin antibodies, IgG and IgM anti- β -2-glycoprotein antibodies, IgG and IgM anti phosphatidylserin antibodies. Hence, the patient re-treated with steroid. Her family asked the follow-up in another hospital.

Conclusion: The low dose immunosuppressive treatment in the patients with LA and hypoprothrombinemia is necessary to continue for the long time

Disclosure of Interest: None declared.

PO009-WED

Achieving haemostatic control in acquired haemophilia a with plasma derived FVIII infusions

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Background: Acquired Haemophilia A (AHA) is a rare and potentially life-threatening disorder characterised by the development of autoantibodies against FVIII. Although bypassing agents (aPCC/rFVIIa) are efficacious in achieving haemostatic control, the thrombotic risk is 2–4% and even higher in patients with pre-existing thrombotic risk fac-

tors. The use of plasma derived FVIII (pdFVIII) concentrate remains an attractive option for managing AHA patients with low titre inhibitors. However, the lack of a consensus and unpredictability of achieving haemostatic control limits its use.

Aims: To describe our experience of pdFVIII concentrate (Fanhdi®) in the treatment of AHA.

Methods: We performed a retrospective analysis of AHA patients treated with Fanhdi® between 2009–2014.

Results: Four patients were identified with median (IQR) age 84 years (81–87). At diagnosis the FVIII:C were 1, 12 iu dL⁻¹, and undetectable in 2 patients with inhibitor levels of 8, 1, 64 and 1000 BU mL⁻¹ respectively. Whilst all patients presented with severe bleeding, they had coexisting cardiovascular disease necessitating cautious use of bypassing agents. Consequently, bypassing agents were stopped and infusions of pdFVIII were started (loading bolus $50\text{--}70 \text{ iu kg}^{-1}$, followed by $8\text{--}10 \text{ iu kg}^{-1} \text{ h}^{-1}$). 1 patient did not receive any bypassing agent. Initial Factor VIII recovery levels varied from 12–70 iu dL⁻¹ and FVIII levels continued to increase in all patients. The duration of the infusion varied from 5–14 days, but all had FVIII levels $> 50 \text{ iu dL}^{-1}$ within 1–21 days of the infusion. Bleeding was well controlled in all with no new thromboembolic events.

Conclusion: Our experience suggests that the use of pdFVIII can limit bypassing agent exposure in AHA. We observed that pdFVIII can achieve haemostatic control in elderly AHA patients with pre-existing thrombotic risk factors, even in the context of high responding inhibitors. Further studies are needed to establish the optimal use of pdFVIII in this setting.

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PO010-WED

Acquired von Willebrand syndrome (AVWS) in patients with cardiovascular disorders: a single centre cohort study

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Background: AVWS associated to cardiovascular disorders (CVD) has been increasingly reported for the past 20 years. Bleeding rate (often major) in these patients, usually under anticoagulant or antiplatelet therapy, may be as high as 80%. Its diagnosis and management is not easy and there are no evidence-based guidelines available. Acute bleeds therapy and hemorrhage prevention during invasive procedures are needed.

Aims: Prevalence evaluation of AVWS in patients with CVD and recent onset of bleeding. Analysis of von Willebrand factor (VWF) profile before and after corrective surgery. Investigation of the therapy employed and impact on the AVWS.

Methods: Haemostasis study including factor VIII (FVIII:C), VWF antigen (VWF:Ag), VWF ristocetin cofactor (VWF:RCO), VWF collagen binding (VWF:CB) and ratios, and VWF multimeric distribution (SDS-agarose gel) was assessed at initial diagnosis, before and after corrective surgery.

Results: Of the 64 patients enrolled, 34 (53%) had AVWS. 28 presented moderate/severe bleeding requiring treatment. Mean VWF:Ag levels were higher than normal. The values of VWF function tests were also high, but lower in patients with AVWS. Mean values of the VWF:RCO/VWF:Ag and VWF:CB/VWF:Ag ratios were < 0.7 in AVWS and close to 1 in patients without AVWS. A loss of high molecular

weight multimers was seen in all AVWS patients. 26 of the 34 AVWS patients were submitted to corrective surgery. 14 were treated with Haemate-P, 1 with rFVIIIr, 1 with tranexamic acid and 10 patients did not received haemostatic therapy. 5 of the patients treated and 4 of the untreated presented surgical bleeding with need of transfusions. One of the untreated required by-passing agents. After surgery, VWF:RCo/VWF:Ag, VWF:CB/VWF:Ag and multimeric analysis were normal.

Conclusion: Diagnosis of AVWS in CVD is difficult and VWF multimeric analysis helps in its confirmation. Surgery of the underlying disorder resolves the AVWS, but these patients face frequently high risk haemostatic challenges.

Disclosure of Interest: None declared.

PO011-WED

Acquired haemophilia a in Poland – a report from AHA-POL registry

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Background: Acquired haemophilia A (AHA) is a rare, potentially life-threatening bleeding disorder caused by autoantibodies inhibiting coagulation factor VIII (FVIII). In 2011 four haematology centres based in four major regions of Poland and covering the population of about 15 million decided to set up a registry to collect data on subjects with AHA.

Aims: To analyse demographic and clinical data on AHA management in the period 2011–2013.

Methods: AHA-POL is a multicentre, web-based registry to collect data on diagnosis, haemostatic and immunosuppressive treatment as well as other aspects of clinical course of AHA, including complications and death rate. Data on consecutive patients from each centre are recorded. Each centre participating in the project is free to use local treatment protocols.

Results: Data on 51 patients (31 male, 20 female, median age 72.5 (range 16–85) were prospectively collected. Ten patients (20%) had concomitant autoimmune disorders and in another 8 (16%) AHA coexisted with malignancy. In 45 (88%) patients the diagnosis was triggered by bleeding episode, and in 35 patients the bleeding was considered major or life-threatening. 37 out of 45 (82%) patients required haemostatic treatment. Only one patient received desmopressin, others were treated with rFVIIIa or aPCC. Both agents were very effective in management of bleeds. Corticosteroids and cyclophosphamide were used in most cases as a first line inhibitor eradication therapy (efficacy about 55%) followed by rituximab and modified immune tolerance regimens. 12 (23.5%) patients died during follow-up. Excessive bleeding was cause of death in 3 (23%) cases; the remaining deaths were associated with concomitant diseases.

Conclusion: Preliminary data from AHA-POL registry indicate that the detection rate of AHA in the studied area is about 1.2 cases per million year⁻¹. The reported high death rate emphasizes the fact that management of bleeding episodes and concomitant diseases in subjects with AHA is a major challenge.

Disclosure of Interest: None declared.

PO012-WED

Successful therapy with bortezomib for eradication of inhibitor in a patient with multiple myeloma and acquired hemophilia A

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Background: Acquired hemophilia A (AHA) is a rare, potentially life-threatening, bleeding disorder. Diagnosis can be difficult and hematological malignancies are one of group-associated diseases.

Aims: To report a case of a patient with multiple myeloma and AHA, with inhibitor eradication with bortezomib.

Methods: An 85-year-old man with a multiple myeloma (G/k) diagnosed 3 years ago, treated with 6 cycles of melphalan and prednisone with low-dose thalidomide maintenance, came to our hospital with abdominal pain, associated with local subcutaneous erythropoietin administration. An abdominal ultrasound (US) and computer tomography scan (CT) revealed an abdominal rectus hematoma, 12 × 9 × 3.5 cm. An AHA was diagnosed through coagulation tests: aPTT 71.8 s (N: 24, 2–36, 4), FVIII level of 1.4%, FVIII inhibitor of 18.4 Bethesda Units (BU).

Results: To achieve bleeding control, he was treated with activated prothrombin complex concentrate (aPCC), and dexamethasone (20 mg, 4 days) plus cyclophosphamide (50 mg day⁻¹) to eradicate inhibitor. On day 5, patient reported pain on right inguinal region and CT showed a new hematoma 19 × 12 × 11 cm in thigh. He maintained treatment and was discharged on day 30 with reduction of hematomas dimension on physical examination and CT. By that time, FVIII level was 2.5% and inhibitor 28.8 BU. Two months later, he returned to hospital with a hematoma in the same location, 14 × 6 cm on US. During 7 days in hospital, he was infused with aPCC and started 4 cycles of bortezomib (1.0 mg m⁻²) and 20 mg dexamethasone/week. After the 1st cycle, inhibitor went down to 0 BU and FVIII level raised to 90%. Actually, after 3 months and 3 cycles of bortezomib completed, FVIII level is in normal range and he has no inhibitor.

Conclusion: There is a consensus that immunosuppression to eradicate inhibitors should be started soon after diagnosis. Data suggest that combination of steroids and cyclophosphamide may result in higher remission rate. In our case, only bortezomib showed to be efficient in inhibitor eradication.

Disclosure of Interest: None declared.

PO013-WED

Critical insights into acquired haemophilia A and risk-benefit aspects with regard to clinical complications: a case report

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Background: Autoantibodies attacking and disabling coagulation factors are associable with high lethality rates. Uncertainty still exists how to treat rare cases with acquired haemophilia. Besides an improvement of the haemostatic balance, inhibitor eradication has to be realized as a second goal.

Aims: The aim is to reach both – effective coagulation as well as immune suppression – which may be rather expensive.

Methods: In a 86 year old patient suspicious for DVT (lower extremity) expanded hematoma and compartment symptoms could be verified. Life threatening bleedings occurred. Red blood cells, fresh frozen

plasma were given, besides prothrombin complex concentrates, platelets, fibrinogen and factor XIII concentrate. Acquired hemophilia A was diagnosed by low levels of factor VIII (10 – 20%), plasma exchange *in vitro*, and the presence of time dependent inhibitor in plasma (2.8 Bethesda Units).

Results: Therapy with recombinant activated factor VII (NovoSeven®) was initiated on day 7 (two to four times daily – adjusted to body weight: 90 micrograms kg⁻¹ BW; totally amount applied: 818 mg). According to algorithms defined by the GTH working group therapeutic options were critically reflected. Immunosuppression was primarily restricted because of wound healing problems. At least (iv) steroids were given, 100 mg daily. Since first line therapy failed immune tolerance therapy was started using rituximab. Clinical improvement became evident 6 to 8 weeks later. Factor VIII increased up to 15% as well as the titre of the autoantibody decreased to 0.5 BU. Lethal outcome due to uncontrollable respiratory distress became unavoidable.

Conclusion: In recent years rituximab proved its beneficial use in the therapy of acquired haemophilia and may in addition to NovoSeven® or activated prothrombin complex concentrates help to reduce high lethality rates in such patients. A big challenge represents the unpredictable costs behind.

Disclosure of Interest: None declared.

PO014-WED

Clinical utility of heat inactivated Nijmegen modified Bethesda assays in FVIII deficiency – a 4 year single centre experience

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Background: Current guidelines state inhibitor assays should be regularly carried out in congenital haemophilia A [HA] patients. Patients with acquired HA require inhibitor tests to monitor treatment. Ideally, FVIII inhibitor tests are most sensitive when FVIII levels have returned to baseline. However, as patients are on prophylaxis, true baseline samples may not be possible to obtain. Presence of FVIII may alter the kinetics of the antigen-antibody reaction and may lead to false negative results. Heat inactivation [HI] of FVIII in patient plasma has been shown to improve sensitivity whilst not affecting inhibitor results.

Aims: To evaluate the clinical utility of the heat inactivated Nijmegen Bethesda assay [NBA] in patients with FVIII deficiency.

Methods: In our centre from 2007–10, no HI was used and samples with FVIII levels > 0.2 iu mL⁻¹ did not have inhibitor tests performed. Since 2011 all samples for inhibitor testing were heat inactivated at 56 °C for 1 h prior to use.

Results: One thousand and eight hundred and thirty-two NBA results from 310 patients during the last eight years were evaluated. 61% (1115) of samples were from patients with severe HA, 22% (399) from mild/moderate HA and 16% (288) from acquired HA patients. Following introduction of HI 95% of samples for NBA were tested (100% in 2014). Prior to 2011, only 72% of samples were tested, as remainder had a FVIII level > 0.2 iu mL⁻¹. Following HI use in NBA, five patients with HA had developed inhibitors, one of which was picked up during laboratory testing [previously a symptomatic patient with decreased baseline FVIII]. 130 samples from 27 acquired HA patients had a FVIII level > 0.2 iu mL⁻¹, and of these 91 were positive with NBA.

Conclusion: Use of HI in the modified NBA allows inhibitor testing in patients receiving prophylaxis facilitating adherence to guidelines. In patients with acquired HA with detectable FVIII levels, it allows for monitoring of the inhibitory antibody, which could be a biomarker to monitor response to immunosuppressive therapy.

Disclosure of Interest: None declared.

PO015-WED

Comparison of different coagulation assays for the management of acquired hemophilia

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Background: Acquired hemophilia (AH) is caused by autoantibodies (inhibitors) to FVIII. The effectiveness of immunosuppressive therapy is often evaluated by inhibitor (Bethesda) and/or FVIII:C levels. However, the Bethesda assay is insensitive to low inhibitor titers (< 0.5 U mL⁻¹) and FVIII:C levels are not sensitive to high inhibitor titers.

Aims: We wanted to determine if alternate coagulation assays might be more sensitive for evaluating responses in AH patients receiving treatment.

Methods: After IRB approval and informed consent, AH patient blood samples were collected at various times during treatment and follow-up. Inhibitor levels were determined by Bethesda assay (BU) and FVIII:C by a one stage clotting assay (Diagnostica Stago). Clotting times (CT) were determined following thromboelastometry (ROTEM) in whole blood. Endogenous thrombin potential (ETP) was determined from thrombin generation (Diagnostica Stago). APTT and second derivative of the clot curves were also analyzed (Instrumentation Laboratory).

Results: Ten AH patients were analyzed over the course of treatment (s) for a total of 96 samples. In 19 samples where the APTT was abnormal (> 37 s) and the BU was < 0.5, the CT was abnormal in 9 of 19 samples, the FVIII in 6 of 19 samples, and the second derivative and ETP in 0 of 19 samples. In 5 samples where APTT was > 37 s and the BU was > 0.5 and < 1, the ETP and second derivatives were abnormal in 1 of 5 samples; 5 of 5 samples had abnormal CT and FVIII:C levels. Regression analysis against BU levels (BU > 0.5 mL) showed good correlation with APTT, CT, ETP and second derivative ($r^2 > 0.4$) when the APTT was < 75 s but poor correlation ($r^2 < .25$) when the APTT was > 75 s (corresponding to approximately 5 BU mL⁻¹).

Conclusion: In AH patients with undetectable inhibitor levels, an abnormal APTT is most likely the result of residual inhibitor. High inhibitor titers (BU > 5 mL⁻¹) are best followed by the Bethesda assay rather than the APTT, CT, ETP, FVIII:C, or second derivative of the APTT clot curve.

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PO016-WED

FVIII/VWF:AG Ratio is a powerful tool to early predict relapse in acquired haemophilia A

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Background: Acquired Haemophilia A (AHA) is a rare bleeding disorder, due to inhibitor against Factor VIII (FVIII). Mortality due to bleedings ranges from 3.5 to 9%. In 50% of cases, AHA is associated with another disease and it is idiopathic in the 50% other cases. Biological diagnosis is based on prolongation of activated partial thromboplastin time (aPTT), low FVIII activity (FVIII:C), and presence of an inhibitor against FVIII. Basic treatment relies on immunosuppressive therapy, especially corticosteroid. There is a high level of relapse in AHA in about 20% of patients. The current criteria of relapse that are decrease of FVIII level above 50% and/or detection of an inhibitor seems to be relatively late.

Aims: We evaluate the ratio FVIII on von Willebrand Factor (FEW ratio) as an early clue to predict AHA relapse and show the example of a patient with a long follow up and a relapse precociously predict by FEW ratio.

Methods: Patients in this retrospective study were diagnosed for an AHA between 1995 and 2012 in Nantes University Hospital or Toulouse University Hospital.

Results: First, we defined normal cut-off of FEW ratio by evaluating this parameter on 40 healthy volunteers. Cut-off was fixed at 0.66 (Mean – 3 SD). We tested 39 patients with inherited hemophilia A, 26 with 2N von Willebrand Disease and 44 AHA. We showed a ratio under the cut-off in all the patients at diagnosis. We then focused on the kinetics of FEW in 27 patients: Twenty-two without relapse and 5 with relapse and showed gradually normalization of FEW ratio during the remission. The first sign of relapse in the five patients was the decrease in the FEW ratio.

Conclusion: We propose that FEW ratio should be integrated in the following of AHA patients. Its decrease or absence of normalization should lead to a narrow clinical and biological monitoring for an early detection of relapse, before bleeding manifestation.

Disclosure of Interest: None declared.

PO017-WED

Assessment of prothrombin time prolongation by thromboelastometry and thrombelastography during liver transplantation

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Background: Prolonged prothrombin time (PT)/INR due to vitamin K-dependent factor deficiency is often exacerbated due to hemorrhage and hemodilution during liver transplantation (1). Viscoelastic coagulation assays including ROTEM and TEG are utilized to monitor the extent of coagulopathy, but their sensitivity to factor deficiency could be affected by different coagulation activators (2).

Aims: The aim of this study was to compare standard ROTEM and TEG assays in evaluating PT/INR prolongation in the setting of acquired vitamin K-dependent factor deficiency during liver transplantation.

Methods: After IRB approval and informed consent, blood samples were obtained for INR, Factor X activity (FX), ROTEM (EXTEM) and TEG (kaolin) from 22 patients undergoing liver transplantation at baseline before surgery, and 30 min after graft reperfusion. Percent changes from the baseline in INR, EXTEM clotting time (CT), and TEG reaction time (RT) after the reperfusion were analyzed by Spearman's correlation (Prism 5, GraphPad, La Jolla, CA). A *P* value < 0.05 was considered statistically significant.

Results: Correlation coefficients between EXTEM-CT and INR (*r* = 0.661) as well as EXTEM-CT and FX (*r* = 0.685) were statistically significant (*P* < 0.001). However, no correlations were found between TEG-RT and INR (*r* = -0.418, *P* = 0.053), and between TEG-RT and FX (*r* = 0.331, *P* = 0.132).

Conclusion: Use of contact activation in kaolin TEG makes it less sensitive to vitamin K coagulation factor deficiency compared to tissue factor-based activation on EXTEM.

Reference: (1) Lu SY, et al. Liver Transplant 2014; 20:1097–1105; (2) Nascimento B, et al. Transfus 2012; 52:7–13.

Disclosure of Interest: S. Lu: None declared, T. Sakai: None declared, E. Abuelkasem: None declared, R. Planinsic Consultant for: Octapharma, K. Tanaka Consultant for: Grifols, Octapharma, TEM.

PO018-WED

A novel diagnostic system of acquired coagulation factor inhibitors using comprehensive coagulation assays

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Background: Acquired coagulation factor inhibitors such as lupus anti-coagulant positive (LA[+]), acquired hemophilia A (AHA) prolong aPTT, but these clinical phenotypes vary from hemorrhagic to thrombosis.

Aims: We attempted to establish quickly differential method of these inhibitors by aPTT-based clot waveform analysis (CWA) and thrombin generation test (TGT).

Methods: Patients' plasmas were obtained from 5 of symptomatic LA (+) (sLA) 10 of asymptomatic LA(+) (non-sLA) and 6 of AHA with hemorrhagic. We performed aPTT-based CWA and TGT, and also TGT with addition of activated protein C (APC).

Results: CWA revealed the prolonged clot time (CT) (NP/sLA/non-sLA/AHA; $30 \pm 2/96 \pm 15/63 \pm 13/116 \pm 44$ s) and the decreased maximal coagulation velocity ([min l] ; NP/sLA/non-sLA/AHA; $3.7 \pm 0.1/2.5 \pm 0.3/1.9 \pm 0.3/1.0 \pm 0.8$), supporting lower coagulation function (*in vitro*) in all groups. TGT also showed the prolonged lag-time (LT) in sLA and non-sLA, and markedly decreased peak-Th in AHA (NP/sLA/non-sLA/AHA; LT: $4.0 \pm 0.3/28.8 \pm 11.8/12.5 \pm 7.7/3.9 \pm 0.7$ min, peak-Th: $370 \pm 33/158 \pm 99/158 \pm 75/55 \pm 35$ nM), indicating that AHA could be distinguished by markedly decreased coagulation function, whilst sLA and non-sLA failed to be distinguished. As one of pathogenic mechanism(s) of LA(+) to discriminate of sLA and non-sLA, APC resistance (APCR) might be raised. APC-induced %inhibition (Normalized % inhibition; 100%) of decrease in peak-Th were lower in sLA: $5 \pm 7\%$ than non-sLA: $42 \pm 39\%$ from patient's plasmas. NP added inhibitor IgGs from patients' plasmas suppressed the APC-induced decrease dose-dependently in sLA and non-sLA ($38 \pm 10/68 \pm 29\%$), and sLA-IgGs showed more significant inhibition compared to non-sLA IgGs, supporting that difference of APCR in both group might reflect the clinical symptoms.

Conclusion: CWA and TGT are useful in differential diagnosis of acquired coagulation inhibitors, and in addition the symptomatic LA (+) might be differentiated from the APCR using TGT.

Disclosure of Interest: None declared.

PO019-WED

Inhibitor of factor VIII in the newborn: case report

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Background: There is no information about inhibitor to F VIII in newborn we find in the available sources. But, theoretically, it is possible, in babies from mother suffer from inhibitor to F VIII.

Aims: Our aim was to describe the case of inhibitor to F VIII in the newborn.

Methods: We observed the newborn girl, who was born from mother, suffer from acquired inhibitor to F VIII. Our observation start at the 8th day after the delivery. Clotting tests was made using coagulometer Sysmex SA1500 and Siemens reagents. Factor VIII activity was analyzed using 1 stage clotting method, and inhibitor was tested, using Nijmegen-modified Bethesda method. Samples collected on 9th, 16th days after delivery, on 1, 5 and 8 month old.

Results: Mother of the girl suffers from acquired coagulopathy due to inhibitor to F VIII with minimal bleeding tendency (easy brushing). Inhibitor was diagnosed during the pregnancy. She had no excessive bleeding after the delivery. No information about titer of inhibitor and medication during and after delivery was available. The girl had no bleeding signs as immediately after delivery, as well as later. The titer of inhibitor to F VIII on the ninth day of life (the first day of observation) was > 20 BU, FVIII $< 1\%$, lupus anticoagulant was negative. She received intravenous immunoglobulin 1 g kg^{-1} two times: at 12th and 13th days of life. After the treatment at 16th day of life inhibitor titer was 43.3 BU, F VIII $< 1\%$. Next test was done in 1 month. No inhibitor activity was detected, F VIII activity = 40.2%. Last observation was provided to the girl in 8 months old. Inhibitor activity = 0, F VIII = 80.9%. No other treatment, except IVIG was provided. The girl had no bleeding symptoms during all period of observation.

Conclusion: It is sample of unusual coagulopathy for newborn. It was interesting the absence of bleeding tendency in children with absence of F VIII activity and high titer of inhibitor.

Disclosure of Interest: None declared.

PO020-WED

Usefulness of various scoring methods with cross-mixing test for detection of lupus anticoagulant

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Background: A cross-mixing test (CMT) of activated partial thromboplastin time (APTT) is used to distinguish lupus anticoagulant (LA) from acquired hemophilia A (AHA) patients. However, this test is dependent on visual judgment of the reaction curve.

Aims: We examined scoring methods for detecting LA with a CMT for improving efficiency.

Methods: Plasma samples from 27 LA and 25 APTT-prolonged patients who received heparin by injection or suffered from hemophilia A were used. CMTs were performed using an automated blood coagulation analyzer with an automatic multi-diluting function (7-point dilution) and APTT, with the following scoring methods. (i) Index of circulating anticoagulant (ICA), calculated using a cut-off of 12.4. (ii) Percent correction (PC), calculated using a positive cut-off value of 18%. (iii) Response curve score (RC-S), calculated using control plasma mixed with 20% and 50% patient plasma samples, and a positive cut-off value of 1.9.

Results: With each scoring method, LA-positive plasma was significantly distinguished from that from patients with coagulation factor deficiency or injected heparin. However, some samples with heparin were false-positive using ICA and many LA-positive plasma samples were false-negative with the PC method. The sensitivity of ICA, RC-S, and PC was 96%, 85%, and 74%, respectively, and specificity was 76%, 100%, and 100%, respectively. ICA and RC-S combined had a sensitivity of 96% and specificity of 100%, and provided a better LA-positive rate than any other scoring method or combination.

Conclusion: ICA showed high sensitivity for detecting LA with a CMT, while PC and RC-S demonstrated high specificity. Our results suggest that the combination of ICA and RC-S is superior to distinguish LA from other APTT-prolonged diseases in CMT findings. ¹Kumano O et al. Clin Lab 2014, 60: 2115–2118; ²Chang S et al. Coag Trans Med. 2002; 117; ³Naito S et al. Rinsho Byori 2012; 60.

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PO021-WED

Defining coagulation dysfunction in patients with leptospirosis

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Background: Leptospirosis is an important public health problem in Sri Lanka, with 3214 cases identified in 2014.

Aims: To evaluate conventional coagulation and rotational thromboelastometry (ROTEM) parameters in leptospirosis patients.

Methods: The study population comprised 20 clinically characterized and serologically confirmed leptospirosis patients (14 severe, 6 mild), 6 patients with dengue, 5 with sepsis admitted to National Hospital of Sri Lanka and 6 healthy individuals. PT, aPTT, TT, Fibrinogen, D-Dimer, Platelet count and ROTEM were performed in all. ANOVA post-hoc comparison using Bonferroni was applied to all parameters.

Results: PT and APTT were prolonged in $> 80\%$ of leptospirosis patients. aPTT corrected with normal plasma confirming deficiency of clotting factor/s. TT was not significantly prolonged in leptospirosis compared with other three groups. Fibrinogen was significantly elevated in severe leptospirosis ($P = 0.001$) and sepsis ($P = 0.001$) compared with healthy controls and dengue. 30% of leptospirosis had thrombocytopenia (17% in mild and 36% in severe), while 100% with dengue were thrombocytopenic and none with sepsis. D-Dimer was elevated in 80% of leptospirosis and (100%) sepsis patients but not in dengue. No significant difference in inTEM CT, and exTEM CT was found in leptospirosis when compared to other 3 groups. inTEM CFT and exTEM CFT in dengue were significantly high compared to severe ($P = 0.001$) and mild ($P = 0.005$) leptospirosis. inTEM MCF ($P = 0.001$), exTEM MCF ($P = 0.001$) and fibTEM MCF ($P = 0.003$) were significantly lower in dengue than in leptospirosis. Only 1 patient with leptospirosis had bleeding manifestations.

Conclusion: Abnormalities in conventional coagulation parameters occur in leptospirosis. However, unlike in dengue, ROTEM parameters in leptospirosis are not significantly altered compared to sepsis and healthy controls. High levels of fibrinogen maybe overcoming the effect of coagulation factor deficiencies and thrombocytopenia in leptospirosis.

Disclosure of Interest: None declared.

PO022-WED

Acquired von Willebrand syndrome – diagnosis and management

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Background: Acquired von Willebrand syndrome (AVWS) is a rare bleeding disorder caused by an acquired deficiency or dysfunction of

von Willebrand factor (vWF). AVWS typically occurs in elderly in association with a variety of diseases, which can cause antibody mediated clearance of vWF, its adsorption to platelets and tumor cell surfaces or proteolysis. Owing to negative history of bleeding and the variability of clinical symptoms the diagnosis and treatment of AVWS are still challenging.

Aims: Report on patients with AVWS treated at our Centre in 2010–2014.

Methods: Patients referred to our centre for unusual bleeding or positive coagulation screening before invasive procedure were tested for primary and secondary hemostasis. For suspected AVWS the examination was extended to comorbidities potentially associated with the syndrome.

Results: AVWS was diagnosed in 7 patients (median age 70; range 14–78 yrs): 3 patients had monoclonal gamopathy (MGUS), 1 M Waldenström, 1 B-CLL, 1 plasmocytoma IgA and a young girl had glycogenosis Ia. The girl had a history of epistaxis and two MGUS pats presented with massive hematuria. Lab results (median, range): FVIII: C 46% (13–80%), vWF:Ag: 25% (12–36%), vWF:RCo: 8% (1–30%), RIPA 17% (6–60%) and PFA closure time > 300. Six and 1 patient had AVWS type 2A and type 1, respectively. Four patients received Haemate with a poor response and rapid drop of vWF:RCo to preinfusion levels in 4–6 h. Oral surgery in 2 pats was successful with Haemate administered in 6 h intervals. Two pats with hematuria received high dose ivIgG resulting in a rapid normalisation of hemostasis in IgGk MGUS, lasting 6 mths. In this patient the ivIgG in 4–5 mths intervals prevents the relapses of AVWS. No remission with ivIgG was achieved in IgMk MGUS, however, transitory improved response to vWF had a clinical effect.

Conclusion: AVWS may be a first symptom of underlying disease. Diagnosis and challenging management of AVWS require close cooperation with experienced hemophilia centres.

Disclosure of Interest: None declared.

PO023-WED

Acquired activated protein C resistance in elderly people with and without cognitive impairment

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Background: In addition to aging, the literature reports the presence of microvascular injury, inflammation and subsequent prothrombotic state, as important factors in exacerbating neurodegenerative process in Alzheimer disease (AD).

Aims: The present study has evaluated the level of acquired resistance to activated protein C (APC) determined with the thrombin generation assay (TGA) in elderly individuals with and without cognitive impairment.

Methods: TGA was determined with the Calibrated Automated Thrombogram-based assay (CAT) in patients with AD ($n = 48$) and in controls without cognitive impairment ($n = 30$). TGA was determined in the presence and absence of APC and the obtained endogenous thrombin potentials (ETP) were used to calculate the normalized activated protein C sensitivity ratio (nAPCsr). Since protein S (PS), tissue factor pathway inhibitor (TFPI) and factor V (FV) are major determinants of thrombin generation determined in the absence as well as the

presence of APC, we also measured their plasma levels with an ELISA (PS or TFPI) or with a prothrombinase-based FV assay.

Results: Considering individuals with nAPCsr > 2 as APC resistant, 17 of the 48 patients with AD (35.4%) were APC resistant while only 2 of the 30 controls (6.7%) had a nAPCsr > 2. FV and TFPI levels were inversely correlated with the nAPCsr *i.e.* with APC-resistance ($r = -0.603$; $P < 0.0001$ and $r = -0.243$; $P = 0.041$, respectively) while PS did not show a correlation. In a multivariate logistic regression model with nAPCsr as dependent variable and FV, TFPI and PS as independent variables, only FV had an independent effect on the nAPCsr values ($P = 0.0001$). No FV_{Leiden} case was detected in this study.

Conclusion: Our study shows that compared to controls, AD patients showed a clear picture of acquired resistance to APC, which may contribute to cognitive decline and dementia. Furthermore, our data suggest an important anticoagulant activity of FV in regulating APC resistance in AD patients. Support: CAPES and CNPq- BRAZIL.

Disclosure of Interest: None declared.

PO024-WED

Single-center experience with rituximab in the treatment of inhibitor-associated hemostatic disorders

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Background: Acquired clotting factor deficiencies are rare but life-threatening bleeding disorders for which Rituximab is used off-label.

Aims: We report a retrospective review of all consecutive cases of acquired coagulation factor deficiency treated at The Ottawa Hospital, contrasting patients who received rituximab and those who did not.

Methods: The Canadian Hemophilia Registry was used to identify patients from 2007 to present. Complete response (CR) was defined as factor activity > 70% and an undetectable inhibitor. Partial response (PR) was defined as factor activity 30–70% and an inhibitor titer decline $\geq 50\%$.

Results: Six patients [4 acquired hemophilia (AH), 1 acquired FXIII deficiency, and 1 acquired VWD] received Rituximab with prednisone $\pm$ cyclophosphamide, and 6 AH patients received prednisone $\pm$ cyclophosphamide alone. In the Rituximab cohort, median baseline factor activity was 4% (range < 1–15%) with a median pre-treatment inhibitor of 26.9 BU mL⁻¹ (range 4.7–538 BU mL⁻¹). Three of the 6 patients exhibited CR and one exhibited PR with a median time to response of 33 days (range 14–69 days). After a median 12 month follow up, two patients relapsed requiring re-treatment, one who re-achieved CR with a second course of Rituximab, and one patient died secondary to FEIBA-associated arterial thrombosis. In contrast, the 6 patients who received prednisone $\pm$ cyclophosphamide alone had less severe bleeding, a lower baseline inhibitor (median 6.3 BU mL⁻¹, range 0.83–30 BU mL⁻¹), and a longer time to response (median 61 days, range 14–107 days). After a median follow-up of 13 months, two of these patients had relapsed, one requiring re-treatment with cyclophosphamide. Numerous adverse events related to the prednisone $\pm$ cyclophosphamide regimen were reported.

Conclusion: Rituximab is effective and well-tolerated for patients with acquired factor deficiencies. Our findings support early Rituximab use to reduce iatrogenic toxicity associated with bypassing haemostatic agents and immunosuppressive therapies.

Disclosure of Interest: None declared.

PO025-WED

Lupus anticoagulant (LA) testing of a pregnant patient revealing an acquired factor XI autoantibodyWool GD¹, Utset TO² and Miller JL¹¹Pathology; ²Medicine, University of Chicago, Chicago, USA

Background: There are rare reported cases of acquired Factor (F) XI inhibitors in patients with systemic lupus erythematosus (SLE); these cases are variably associated with bleeding.

Aims: We report discovery of an acquired FXI circulating inhibitor during LA testing in a pregnant patient with SLE.

Methods: Instruments and reagents used: STA-R Evolution, STA-PTT Automate, PTT-LA, Cryocheck Platelet Lysate (Precision BioLogic), Thermo Scientific Nab protein G and Zeba desalting columns, Cobas 8000 immunoglobulin quantitation.

Results: A woman in her 30 s, diagnosed with SLE 14 years previously, had tested negative for an LA (including normal PTT-LA) 6 and 4 years previously. The patient had no personal or family history of bleeding. Current prenatal testing for functional LA showed normal DRVVT and dilute PT but evidence of a non-phospholipid-dependent inhibitor in the PTT-LA: the screen was markedly prolonged, the mix failed to correct, and the confirmatory phase showed no significant correction. Further testing for FVIII, FIX, FXI, and FXII levels revealed an isolated severe FXI deficiency (< 1%, normal 78–133%) with a circulating FXI inhibitor (3.0 Bethesda Units [BU]). The factor XI inhibitor titer peaked at 4.1 BU at 18 weeks gestation. Plasma from that time point was completely depleted of detectable inhibitory activity by treatment with protein-G agarose beads (post-bead plasma FXI inhibitor titer < 0.6 BU). The eluted material showed inhibitory activity (1.6 BU, or 84% of expected recovery), confirmed to be IgG. The patient was treated with immunosuppressants. Subsequent investigations of FXI and inhibitor showed continued low FXI (3–6%), but without detectable circulating inhibitor.

Conclusion: The cause of this patient's persistently low FXI level despite eradication of detectable circulating inhibitor is unclear at this time; a mechanism involving persistent non-circulating immunoglobulin inhibitor cannot be excluded.

Disclosure of Interest: None declared.

PO026-WED

Acquired thrombotic thrombocytopenic purpura (TTP) in patient with heterozygous MTHFR C677T mutation and hyperhomocysteinemia: a case reportCeglarek B¹, Bykowska K², Wiszniewski A³ and Windyga J¹¹Department of Disorders of Hemostasis and Internal Medicine;²Department of Hemostasis and Metabolic Disorders;³Department of Vascular Surgery, Institute of Hematology and Transfusion Medicine, Warsaw, Poland

Background: Thrombotic thrombocytopenic purpura (TTP) is an autoimmune disorder. Idiopathic TTP is caused by acquired autoimmune deficiency of a plasma metalloprotease ADAMTS13 and may be associated with cancer, some drugs, infections or tissue transplantation.

Aims: Acquired TTP in patient with heterozygous MTHFR C677T mutation and hyperhomocysteinemia

Methods: Patient in poor general condition (ECOG 4), conscious without contact, no hemorrhagic diathesis, no lymphadenopathy and hepatosplenomegaly. There was observed one episode of transient brain ischemia attack in the amplitude of vascularized of right and left internal carotid arteries with transient right and left-sided hemihypaesthesia with symptoms: confusion, no logical contact, psychomotor agitation. Lab tests showed: WBC $9.4 \times 10^3 \text{ L}^{-1}$, RBC $1.7 \times 10^6 \text{ L}^{-1}$, Hb 5.7 g dL⁻¹, HCT 17.0%, platelet $7.0 \times 10^3 \text{ L}^{-1}$, RBC-anisocytosis, poikilocytosis, mikrocytosis, makrocytosis, schisto-

cytes, reticulocytes: 9.03%, hypercholesterolemia, hyperlipidemia, antinuclear antibodies (ANA3), lupus anticoagulant (LA) were not detected, heterozygous mutation MTHFR C677T and hyperhomocysteinemia (20.90 $\mu\text{mol L}^{-1}$), ADAMTS 13 activity < 2% and ADAMTS13 inhibitor 41 U mL⁻¹.

Results: The decompensated arterial hypertension and chronic nicotine were observed. The therapy with 27 plasmapheresis utilize FFP (after 2-nd procedure 1 epileptic attack Grand-Mal was maintained), steroids (Prednisone, Methyl-Prednisolone), red blood cell infusions-2 units were administered for 4 weeks. During therapy ADAMTS13 activity was increased from 2 to 62% and ADAMTS 13 inhibitor decreased from 41 to 12 U mL⁻¹. Remission was achieved for last 34 months.

Conclusion: Patients with severe clinical manifestations of TTP, heterozygous MTHFR C677T mutation, hyperhomocysteinemia, hypercholesterolemia, hyperlipidemia and uncontrolled arterial hypertension can be successfully treated with using plasmapheresis and steroids.

Disclosure of Interest: None declared.

PO027-WED

Acquired inhibitors to factor VIII AND IX, a case report

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Background: Acquired hemophilia (AH) is a rare autoimmune bleeding disorder caused by antibodies to coagulation factors, most commonly to factor (F) VIII. Up to 50% of cases are idiopathic.

Aims: A patient with high FVIII and concomitant FIX inhibitors is presented.

Methods: Activated partial thromboplastin time (APTT) and prothrombin time, with mixing studies were performed. FVIII, FIX, FXI and FXII levels were measured by one-stage APTT-based assay. Dilute Russell's Viper Venom Time test for lupus anticoagulants (LA) was carried out. Bethesda assay was used to quantify coagulation factor inhibitors. Additionally, anti-FVIII and anti-FIX enzyme-linked immunosorbent based assay (ELISA) was performed.

Results: A 59-year old, previously healthy female patient was admitted for bleeding diathesis after trauma surgery. In the screening tests APTT was prolonged; mixing studies revealed inhibitors. FVIII and FIX levels were both under 0.01 IU mL⁻¹ with inhibitor titers of 358 BU mL⁻¹ and 6 BU mL⁻¹, respectively. LA were excluded. FIX inhibitors were negative by anti-FIX ELISA test. Bleeding was controlled with recombinant activated FVII, at the same time immunosuppression (methylprednisolone and cyclophosphamide) was started. Immunosuppression was discontinued 4 months later because of post-operative wound infection. At that time FVIII level was 0.02 E mL⁻¹ and inhibitor titer 2 BE mL⁻¹. With the intention to prepare the patient for removal of osteosynthesis material 3 years later, rituximab was given, which ultimately led to complete eradication of inhibitors. A year later she died of disseminated small cell lung cancer.

Conclusion: Acquired inhibitors to coagulation factors other than FVIII are extremely rare. Decreased FIX activity in our case was probably an *in vitro* artifact caused by depletion of FVIII by high titer of FVIII inhibitor. Premalignant state could hypothetically be the reason for AH, on the other hand malignancy could be facilitated by immunosuppression therapy.

Disclosure of Interest: None declared.

PO028-WED

Antitrombin III function in infection development in patients with severe burns

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*Federal State Budgetary Institution 'Privolzhsky Federal Medical Research Center' of Ministry of Health of Russia, Nizhny Novgorod, Russia***Background:** Antitrombin III (ATIII) is one of the significant inhibitors of blood clotting system having both anticoagulative and anti-inflammatory effect.**Aims:** Study the role of AT III deficiency in the acute period of burn disease in patients with hard burning injuries.**Methods:** The state of coagulative, anticoagulative and fibrinolytic chains of the hemostasis system, as well as biochemical blood metabolites, indicative of the functional state of kidneys and liver, were examined in 311 patients with burns. The burn disease was accompanied by infectious complications - sepsis and pneumonia (33 and 67 patients respectively) in 100 patients.**Results:** The analysis of the results obtained showed that 70% of patients (311/218) with severe burn injuries in the acute period of burn disease had decreased activity of AT III. ATIII activity correlated with albumen concentration ($r = 0.42 - 0.64$, $P < 0.0001$) on the 1st-10th day and cholesterol ($r = 0.4 - 0.52$, $P < 0.001$ on the 2nd-10th day), neutrophil elastase activity ($r = -0.49$, $P < 0.02$) on the 1st-2nd day, thrombocyte number ($r = 0.35 - 0.52$, $P < 0.001$) on the 2nd-10th day. The highest diagnostic value of ATIII deficiency in development of purulent-septic complications of the burn disease (sepsis and pneumonia) was revealed from the 3 days to the 7th day of the burn ($\chi^2 = 21.6 - 6.63$, $df = 2$; $P < 0.005$). It should be noted that ATIII activity reduction preceded establishing diagnosis of sepsis and pneumonia in the hospital from 2 to 8 days.**Conclusion:** AT III deficiency is not only a characteristic feature of the acute period of the burn disease in patients with a severe thermal injury, but also a diagnostic and prognostic criterion of development of infectious complications - sepsis and pneumonia, in patients with the burn disease.**Disclosure of Interest:** None declared.

PO029-WED

Efficacy of FVIII in the treatment of patients with acquired haemophilia A and vascular diseasesPasca S¹, Battello C² and Barillari G¹¹Center for Hemorrhagic and Thrombotic Diseases; ²Medicine Department, University Hospital of Udine, Udine, Italy**Background:** Acquired Haemophilia A (AHA) is a severe, rare and potentially life-threatening bleeding that affects males and females, often secondary to other previous diseases. Guidelines recommend early treatment with bypassing agents and, if these products are not available or the anti-FVIII < 5 BU, with FVIII. Inhibitor eradication should be performed with corticosteroids and/or cyclophosphamide. But in case of patients with vascular diseases with thromboembolic risk, when bypassing agents are contraindicated which is the best strategy for AHA?**Aims:** To evaluate the efficacy of FVIII in the treatment of patients with AHA, vascular diseases and anti-FVIII > 5 BU**Methods:** Two patients with vascular diseases and AHA referred to our center and treated with FVIII, corticosteroids and/or cyclophosphamide without any use of bypassing agents**Results:** First patient, 70 year old man, with previous atrial fibrillation, aneurysm middle cerebral artery, renal failure, chronic obstructive pulmonary disease admitted to hospital with ileo-psoas haematoma, FVIII 0.4%, anti-FVIII 32.3 BU. Treated initially with FVIII 4000 IU day⁻¹ followed by 3000 IU day⁻¹ and corticosteroid ther-

apy. One month later FVIII 24.2%, anti-FVIII 2.2 BU. Three months later FVIII 49%, inhibitor disappeared. No adverse events reported. Second patient, 71 year old man, with previous acute myocardial infarction and consequent coronary artery by-pass graft and vascular cervical myelopathy admitted to hospital with serious haemorrhagic muco-cutaneous syndrome, FVIII 2.5%, anti-FVIII 28 BU. Treated initially with pdFVIII 4000 IU per bid followed by 3000 IU per bid, corticosteroid therapy and cyclophosphamide. One month later FVIII 40%, anti-FVIII 1.1 BU. Four months later FVIII 64%, inhibitor disappeared. No adverse events reported

Conclusion: In case of patients with vascular disease at high risk of VTE, in which use of bypassing agents is contraindicated, FVIII can be a valid, safe and effective alternative for the treatment of AHA**Disclosure of Interest:** None declared.

PO030-WED

Correlation between indices of hemostasis system and biochemical blood metabolites in patients with diabetic foot syndrome

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*Federal State Budgetary Institution 'Privolzhsky Federal Medical Research Center' of Ministry of Health of Russia, Nizhny Novgorod, Russia***Background:** Diabetic foot syndrome (DFS) is one of the latest and hardest complications of diabetes mellitus.**Aims:** Study the relation between hemostasiological and metabolic changes in patients with a neuroischemic form of diabetic foot syndrome.**Methods:** The state of coagulative, anticoagulative and fibrinolytic chains of the hemostasis system, aggregative thrombocyte activity and biochemical blood metabolites were examined in 15 patients with a neuroischemic form of DFS. The control group included 50 apparently healthy persons. Statistical data processing was made with methods of descriptive statistics, sample comparison (Mann-Whitney U test) and correlation analysis (Spearman correlation coefficient).**Results:** The analysis of results obtained showed that patients with a neuroischemic form of DFS along with changes in carbohydrate, protein and lipid metabolism, and inflammatory response developed marked hemostasis system disorders: hypercoagulation, thrombocyte hyperaggregation, high thrombinemia level, fibrinolysis suppression, endothelium dysfunction. The research revealed correlation between hemostasis results and biochemical blood metabolites. The level of CRP correlated with the fibrinogen concentration ($r = 0.67$, $P < 0.05$), von Willebrand factor activity ($r = 0.76$, $P < 0.001$), antithrombin III ($r = -0.52$, $P < 0.05$), thromboelastogram profile ($r = 0.66 - 0.82$, $P < 0.02$). There was a correlation between albumen content and XIIa-dependent activation of fibrinolysis (XIIa-DF) ($r = -0.76$, $P < 0.001$), von Willebrand factor ($r = -0.74$, $P < 0.001$), thromboelastogram profile ($r = -0.69 - -0.85$, $P < 0.02$). A correlation between HDL and fibrinogen concentration ($r = -0.69$, $P < 0.03$), antithrombin III activity ($r = 0.7$, $P < 0.03$), XIIa-DF ($r = -0.72$, $P < 0.02$), thromboelastogram profile ($r = -0.67 - -0.69$, $P < 0.05$) was revealed.**Conclusion:** Changes in hemostasis system have a high degree of correlation with development of inflammatory and metabolic disorders in patients with a neuroischemic form DFS.**Disclosure of Interest:** None declared.

PO031-WED

Neutralizing autoantibody against factor XIII a subunit with multiple inhibitory effect on factor XIII activation/activity resulted in severe bleeding with fatal outcome

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Background: Autoantibodies may develop against the catalytic A subunit of factor XIII (FXIII-A) or the carrier B subunit (FXIII-B). Both types are associated with severe bleeding diathesis. Anti-FXIII-A antibodies can be sub-grouped as neutralizing, non-neutralizing or combined. Neutralizing antibodies may inhibit thrombin and/or Ca²⁺ induced FXIII activation (type I), the activity of FXIIIa (type II), FXIIIa-fibrin interaction (type III) or may exert multiple effects (type IV).

Aims: After autoimmune FXIII deficiency of an elderly (75) patient with severe bleeding symptoms had been diagnosed we aimed to characterize the autoantibody.

Methods: Plasma FXIII activity was measured by the ammonia release assay, FXIII-A₂B₂, FXIII-A and FXIII-B antigen were determined by ELISA. The binding of patient's IgG to plasma FXIII and to FXIII-A₂ was tested by surface plasmon resonance (SPR). The presence of inhibitor was verified by mixing study and its titer was estimated by Bethesda assay. The effect of patient's IgG on the thrombin cleavage of FXIII-A was demonstrated by Western blotting, while the effect on Ca²⁺ induced activation and on activated FXIII was measured by activity assay.

Results: FXIII activity, FXIII-A₂B₂ and FXIII-A antigens were below the limit of detection in the patient's plasma; platelet FXIII activity and FXIII-A antigen were normal. Mixing study indicated the presence of inhibitor with a titer of 63.2 Bethesda unit (BU). The patient's IgG bound to FXIII-A₂B₂ and to FXIII-A₂ with equally high affinity (K_d ≈ 10⁻⁸ M). It showed a multiple inhibitory effect on FXIII activation/activity (IC₅₀ was 50 µg mL⁻¹). Immunosuppressive therapy gradually decreased the titer to 8.0 BU, but FXIII level remained very low and due to recurrent bleedings the patient passed away.

Conclusion: Autoimmune FXIII deficiency is a severe bleeding diathesis, which is difficult to manage. In our case a high titer of autoantibody with high affinity toward FXIII-A exerted multiple inhibitory effect and lead to fatal bleeding.

Disclosure of Interest: None declared.

PO032-WED

The acquired deficiency of factors II, VII, IX, X in the course of the intoxication of second generation anticoagulant rodenticides in female patient suffering with severe diathesis haemorrhagica – a case report

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Background: Second generation anticoagulant rodenticides (superwarfarins) and warfarin containing rodenticides are used for rodent extermination. Severe poisonings in humans can be caused by failure to adhere to safety principles, oral use and rarely by transdermal contact. They cause a marked reduction in the levels of the vitamin-K depen-

dent factors II, VII, IX, X while factors V and VIII:C are within normal range.

Aims: The acquired deficiency of factors II, VII, IX, X in the course of the intoxication of second generation anticoagulant rodenticides in patient suffering with severe diathesis haemorrhagica

Methods: Female came into direct contact with superwarfarins a few months earlier but denied having taken anticoagulant rodenticides orally. History: epilepsies, scoliosis, hiatus hernia, mammary cysts, glioma and post operation hematoma extirpation and reactive arthropathy of right shoulder joint, both wrists, knee and tarsal joints. The patient was admitted for severe hemorrhagic diathesis (metrorrhagia, epistaxis) lasting for 1 month, no lymphadenopathy and hepatosplenomegaly were observed. The laboratory tests showed: the prothrombin time > 120.0 s, INR > 12.0, prothrombin index < 8.0%, thrombin time-15.8 s, APTT-87.5 s, factor II-14.0%, factor V activity-116.0%, factor VII activity-2.0%, factor VIII activity-159.0%, factor IX activity-2.8%, factor X activity-3.0%, fibrinogen-5.5 g L⁻¹, antigen of factor von Willebrand-214.0%, factor von Willebrand's activity-184.0%. Lupus anticoagulant was not present, correction time was negative.

Results: Therapy with fresh frozen plasma (FFP) at 10–15 mL kg⁻¹ dose for 3 days: two times in 16 days and vitamin K at 10–20 mg i.v. per 24 h dose allowed to achieve clinical improvement: severe hemorrhagic diathesis was eliminated, metrorrhagia, epistaxis stopped.

Conclusion: Severe bleeding after the superwarfarins intoxication can last months even up to 3 years. Therapy with FFP, vitamin K is effective and minimizes morbidity and mortality.

Disclosure of Interest: None declared.

PO033-WED

Prediction of bleeding complications after renal biopsy in post-renal transplant patients with uremia

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Background: Patients with renal failure and uremia are thought to have an acquired bleeding tendency due to trombocyte dysfunction. Frequently, they are undergoing renal biopsies, making them prone for a post procedure bleeding. Current recommendations for assessing bleeding tendency prior to biopsy are history taking and laboratory testing of biochemical profile, complete blood count, platelet count, prothrombin time, and partial thromboplastin time and bleed- ing time.

Aims: In this study we aimed to look in high uremia patients post renal plantation awaiting renal biopsy whether relevant postprocedural bleeding exist and could be predicted using combinations of hemoglobin and trombocyte counts, bleeding time according to Ivy, area-under-curves (AUC) of multiple electrode aggregometry (MEA), and closure times (CT) of PFA-100.

Methods: A single center prospective observational cohort study. All post-transplant patients for renal biopsy were included with ureum > 10 mmol L⁻¹. Biopsy was done with an ultrasound guided 16 gauge needle. Endpoints were: need for transfusion, hemoglobin drop more than 1 mmol L⁻¹, and postbiopsy ultrasound findings of bleeding.

Results: Ninety patients were included in this study. Five patients had a hemoglobin drop over 1 mmol L⁻¹. One patient needed transfusion after the biopsy for a bleed on ultrasound. All high bleeding times (n = 5) were false-positive. Similarly, all prolonged CT's using the epinephrine cartridge of PFA-100 did not predict for bleeding. One patient with an endpoint had a prolonged CT on the ADP cartridge. In MEA-testing high AUC's had a negative predictive value of 100%,

whereas all patients with an endpoint showed a low MPV (positive predictive value 10%). Hemoglobin, thrombocyte, ureum, or creatinine levels prebiopsy did not differ significantly between patients with or without an endpoint.

Conclusion: Bleeding complications are low after renal biopsy in post-transplant patients with uremia. Bleeding could not be predicted, but safely excluded if MEA testing gave high results.

Disclosure of Interest: None declared.

PO034-WED

Analysis of the profile of immune cells, cytokines and chemokines in previously untreated patients with hemophilia

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Background: The development of inhibitors is the most serious complication in patients with hemophilia. The physiopathology of inhibitors is poorly understood and few studies have focused on the immunological environment of patients with hemophilia.

Aims: To evaluate the immunological profile of previously untreated patients (PUPs) with hemophilia before starting replacement with deficient factor.

Methods: PUPs are followed prospectively with collection of socio-demographic, clinical and laboratory data. PUPs were evaluated for leukocyte cell activation markers. PUPs and controls were evaluated for cytokines and chemokines by flow cytometry. The control group was composed of healthy, non-hemophilic boys. All parents signed a written informed consent.

Results: A total of 34 patients were included, of whom 29 (85.2%) with hemophilia A and 5 (14.7%) with hemophilia B. Sixteen patients (47%) were evaluated for cell activation markers according to the expression of CD4⁺HLA-DR⁺, CD8⁺HLA-DR⁺, CD3⁺CD16⁺CD56⁺, CD3⁺CD16⁺, CD5⁺CD19⁺, CD19⁺HLA-DR⁺, CD14⁺HLA-DR⁺, CD16⁺HLA-DR⁺, CD19⁺CD32⁺, CD19⁺CD20⁺, CD19⁺CD27⁺, CD19⁺CD80⁺, CD86⁺CD19⁺CD86⁺, CD64⁺CD14⁺CD64⁺ and CD4⁺CD25⁺. Tests for cytokines INF- γ , TNF, IL-17, IL-10, IL-6, IL-4 and IL-2 and chemokines IL-8, RANTES, MIG, MCP-1 and IP-10 were performed in 29 patients (85.2%) and 47 controls. Significant higher concentration of TNF ($P = 0.032$), IL-10 ($P = 0.001$), IL-6 ($P = 0.005$), IL-4 ($P < 0.001$), IL-2 ($P = 0.032$) and IL-8 ($P < 0.001$) was observed in PUPs when compared to the control group. We also found a reduction in MIG in PUPs compared to the control group ($P < 0.001$).

Conclusion: Leukocyte activation profile differs in newly diagnosed patients. The immunological profile modulated by cytokines and chemokines differs in PUPs when compared to healthy boys. The significance of these findings will be further elucidated by prospective follow-up of the immune profile of hemophilic patients with regard to the development of inhibitors.

Disclosure of Interest: None declared. This study was supported by Fapemig and CNPq.

PO035-WED

Prospective validation of clini-biological parameters including initial hemostasis, which improve the prediction of death at 1 month among patients with septic shock: Sepsicoag study

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Background: We proposed a prognostic score, based on retrospective data obtained in the first 24 h of routine clinical/biological practice management, to predict death at 1 month and at the end of the first week in patients with septic shock.

Aims: The main objective of this multicenter study is to validate the performance of the score in terms of area under the ROC curve and negative predictive value. Secondary objectives: (i) validate a prognostic score for death at the end of the first week from initial data. (ii) Validate a predictive score of disseminated intravascular coagulation (DIC) in patients with septic shock by comparing with the Gold Standard of the International Society on Thrombosis and Haemostasis (overt-DIC in the first 5 days after inclusion). These scores are based on biological markers scored under emergency situations within the first 24 h of routine clinical practice management for septic shock.

Methods: Seven hundred eighty consecutive patients with septic shock entering in seven institution's ICU in France were investigated on the first day of admission, and deaths were registered at the end of the first week and during the first month. Data recorded at admission included age, sex, referring facility, primary and secondary admission diagnoses, and associated comorbidities. Treatments used within the first month were systematically recorded. All of the patients were continuously monitored for 60 days after they had entered the ICU. The SAPS-II score and the SOFA score were obtained within 24 h of admission and at D1, D3, and D5.

Results: To date, recruitment, assays, encoding data and data monitoring have been completed, statistical analysis is underway.

Conclusion: Final results will be sent for the late breaking clinical trials submission deadline.

Disclosure of Interest: None declared.

ADAMTS13 II

PO036-WED

The importance of conformational activation of ADAMTS13 for control of platelet deposition under flow

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Background: We recently reported that ADAMTS13 activity against VWF is dependent on a conformational change induced by binding to the D4CK domains of globular VWF. The inactive ADAMTS13 conformation is maintained by an interaction between the spacer and CUB domains and is disrupted by the introduction of spacer domain mutations in the ADAMTS13 gain of function (GoF) variant R568K/

F592Y/R660K/Y661F/Y665F. The pathophysiological implications of conformational activation have yet to be fully explored.

Aims: We aimed to establish whether the function of ADAMTS13 against VWF dependent platelet deposition under flow is influenced by conformational activation and, if so, whether the pre-activated state of the GoF variant results in an increased inhibition of platelet deposition.

Methods: ADAMTS13 and its GoF variant were expressed at high levels in HEK293S stable cell lines. The influence of ADAMTS13 on VWF mediated platelet capture and clot lysis was determined using collagen coated laminar flow chambers.

Results: Flow assays using washed platelets at varying ADAMTS13 concentrations (0.25 – 10 nM) established EC_{50} values of 0.48 ± 0.06 nM and 0.13 ± 0.06 nM, $n = 3$, for WT and GoF ADAMTS13, respectively, suggesting a ~3 fold enhanced activity similar to static assay data. However, using clot lysis assays the proteolytic activity of GoF ADAMTS13 was enhanced ~10 fold, with EC_{50} values of 13.2 ± 0.08 nM and 1.67 ± 0.66 nM, $n = 3$, for WT and GoF ADAMTS13 respectively. Pre-activation of WT ADAMTS13 by VWF-D4CK resulted in an increase in activity in flow assays, to an extent similar to that of the GoF variant.

Conclusion: Conformational activation of ADAMTS13 by VWF D4CK under flow, resulting in inhibition of VWF dependent platelet deposition, is not required in the GoF variant which already adopts this conformation and is pre-activated. We are currently investigating if the enhanced activity of GoF variant can provide protection in $FeCl_3$ induced thrombosis *in vivo*.

Disclosure of Interest: None declared.

PO037-WED

The impact of timely ADAMTS13 results on clinical diagnosis and management of patients with suspected thrombotic microangiopathy (TMA): mayo clinic's experience

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Background: Thrombotic thrombocytopenic purpura (TTP) is due to congenital or acquired deficiency of von Willebrand factor cleaving protease ADAMTS13 (AD13). TTP is a clinical diagnosis, but severe AD13 deficiency ($\leq 10\%$ activity) is specific for TTP in contrast to other TMA disorders. Early recognition and initiation of therapeutic plasma exchange (TPE) reduces mortality and prompt availability of AD13 ensures judicious use of TPE. In 2012, we implemented the AD13 assay with a < 24 h turnaround time.

Aims: Assess the impact of timely AD13 results in diagnosis and management of TMA.

Methods: Retrospective study (9/2012-9/2014) of consecutive pt with clinical suspicion of TMA and pre-TPE AD13 results. Review of clinical findings, laboratory data, TPE treatments (duration and units of plasma used).

Results: One hundred and six eligible pt (116 AD13 assays) met our study criteria, (females, 58.5%, median age 57 yrs, range 1–93 yrs). 28/106 (26%) received empiric TPE. 7/106 (7%) pt with high clinical probability of TTP responded to a mean of 9 TPE procedures (range 5–21); 21/106 (20%) of the remaining pt received empiric TPE; 6/21 TPE was stopped (range 1–4 procedures) based on normal AD13 activity and alternative explanation for TMA. The mean AD13 activity for the 7 TTP pt was 26% (range 5–76%) of which 3 had positive inhibitor screen (titers 1.3, 1.3 and 2.7 Bethesda Units). In contrast, mean AD13 activity for non-TTP pt ($n = 99$) was 71% (range 15–100%) ($P = 0.003$). Pt who had no TPE ($n = 81$), and pt who had TPE discontinued ($n = 6$) showed a significantly higher AD13 activity

(71%, 79%, respectively), higher platelet count (105 ($P = 0.001$) and 80 ($P = 0.04$) vs. $36.5 \times 10^9 L^{-1}$, respectively) and higher creatinine (2.5 ($P = 0.005$) and 6.3 ($P = 0.007$) vs. 1.4 mg dL⁻¹, respectively) than likely TTP pt.

Conclusion: Timely availability of AD13 results combined with clinical findings facilitated efficient diagnosis and judicious use of TPE in management of patients with suspected TMA.

Disclosure of Interest: None declared.

PO038-WED

ADAMTS13: von Willebrand factor cleaving protease. ... growth factor?

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Background: ADAMTS13 has been recently identified as a negative regulator of the pro-angiogenic growth factor, VEGF. In the absence of VEGF, ADAMTS13 also functions as a positive regulator of angiogenesis by increasing cell migration and proliferation in HUVECs. Though it was observed that ADAMTS13 induces VEGFR2 phosphorylation, it is not known if this effect is direct or indirect, or if ADAMTS13 induces the same signaling cascades as VEGF.

Aims: Investigate the pro-angiogenic mechanism of ADAMTS13. Hypothesis: ADAMTS13 functions through a CD36:VEGFR2 protein complex to activate pro-angiogenic signaling pathways resulting in gene up-regulation and signal attenuation through degradation of CD36 and VEGFR2.

Methods: All experiments utilized primary HUVECs. Western blots were used to determine protein concentration of AKT, phospho-AKT (Ser473), CD36, ERK1/2, phospho-ERK1/2, VEGFR2, and GAPDH (control). Total RNA was purified from HUVECs treated with ADAMTS13 (150 ng mL⁻¹). Real time-PCR was performed, and relative gene expression was determined by the Pfaffl method.

Results: (i) ADAMTS13 increased phosphorylated AKT and ERK after a 10 min incubation period. (ii) HUVECs incubated with ADAMTS13 over 120 min showed a decrease in total CD36 and VEGFR2 protein. (iii) of the six immediate-early genes investigated, the mRNA levels of three increased more than two fold (EGR2/FOS/NR4A2), and 2 genes had a minor increase (DUSP5/DUSP6) after ADAMTS13 incubation.

Conclusion: ADAMTS13 incubation with HUVECs activates the AKT and ERK signaling pathways, induces degradation of CD36 and VEGFR2, and up-regulates the immediate early genes, EGR2, FOS, NR4A2, DUSP5, and DUSP6. These data suggest that ADAMTS13 is an endothelial cell growth factor which acts through a CD36:VEGFR2 protein complex.

Disclosure of Interest: None declared.

PO039-WED

Detection of ADAMTS13 activity using the native VWF91 peptide

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Background: Many methods based on the short VWF peptide have been developed to detect ADAMTS13 activity, such as FRET-VWFs based on the donors and quenchers or based on homo self-quenchers. Mutations were introduced into those peptides although the fluorescence quenching might be different, especially the efficacy of homo self-quenchers is uncertain. Meanwhile, the elements such as bilirubin or free hemoglobin in plasma interfere the detection of ADAMTS13 using the above peptides.

Aims: To set out to produce the native VWF91 peptide labeled with one single fluorescein-5-maleimide (F5M) to detect the ADAMTS13 activity in plasma.

Methods: The bacterial preference codon sequence of VWF91 (G1579-C1669) was synthesized and inserted into the plasmid pQE30, meanwhile, the TEV recognition sequence of ENLYFQ was added directly into the N terminal VWF91. The plasmid was transferred into the *E. Coli* M15 and the fusion peptide was expressed after induced by IPTG. The fusion peptide was purified by Ni column followed by adding F5M. After the free F5M was washed away, the fusion peptides were eluted from the column followed by the cleavage by TEV. The labeled VWF91 was achieved by purification of the mixture by R-HPLC, and then incubated with serially diluted normal plasma or with plasma from patients with acquired TTP for 1 h under 37 °C, and the reaction was stopped by adding reducing loading buffer and subjected to SDS-PAGE (15% gel). The band intensities of the reaction products were visible under the UV stimulation-fluorescence image and the relative activities of patients' plasma were calculated.

Results: The soluble VWF91 peptide was expressed and the ADAMTS13 activities from 9 acquired TTP patients were < 3% using the labeled VWF91 as the substrate.

Conclusion: The ADAMTS13 activity in plasma can be detected using the labeled intact VWF91 without interference such as bilirubin, and it is easy to perform the assay within 2.5 h.

Disclosure of Interest: None declared.

PO040-WED

Characteristics and outcomes of patients with upshaw-schulman syndrome receiving maintenance hemodialysis due to chronic renal failure

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Background: Thrombotic thrombocytopenic purpura (TTP) is now well known to be caused by deficiency of ADAMTS13 activity (:AC) due to *ADAMTS13* gene mutations (Upshaw-Schulman syndrome: USS) or ADAMTS13 autoantibodies in acquisition. Among patients with acquired TTP, renal dysfunction is usually mild and reversible. When severe renal dysfunction is predominant feature, the disorder is suspected to be hemolytic uremic syndrome (HUS). However, chronic renal failure is sometimes found in patients with USS.

Aims: To clarify the characteristics and outcomes of patients with USS who received maintenance hemodialysis due to chronic renal failure.

Methods: We identified 57 patients with USS in Japan (33 females and 24 males). USS was diagnosed by severe deficient ADAMTS13:AC (< 10%) and negative for ADAMTS13 inhibitor. Of these, 6 patients developed severe chronic renal failure requiring maintenance hemodialysis despite of receiving plasma therapy. The diagnoses of USS were confirmed by *ADAMTS13* gene analysis except for one patient. Patients with short time hemodialysis due to acute renal failure were excluded.

Results: All six patients were males and had no episodes of severe neonatal jaundice requiring exchange blood transfusion. The ages of year at initiation of dialysis were between 22 and 52. The intervals from the diagnosis of TTP or HUS to dialysis were between 1 and 25 years. One patient was diagnosed with USS 10 years later from the initiation of dialysis. Surprisingly, 5 out of 6 patients were died after 6 months to 19 years of time on dialysis. Fifty-one USS cases without severe chronic renal failure had 3 dead cases and each one of sudden death, pregnancy, and cerebral infarction.

Conclusion: We found that all patients with maintenance hemodialysis were males and had no episodes of severe neonatal jaundice. Many

death cases of USS were observed in patients with severe renal dysfunction who were required maintenance hemodialysis. Therefore, therapy of USS should aim to reduce the risk of chronic renal failure.

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PO041-WED

The course of ADAMTS13-properties during pregnancy induced thrombotic thrombocytopenic purpura (TTP)

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Background: It is well known that pregnancy can precipitate thrombotic thrombocytopenic purpura (TTP) with severe maternal and fetal complications. We present our data from the seventh pregnancy week until up to now 3 month after birth of a healthy full-term child. The 31 years old patient suffered from three cerebellar infarctions until the seventh week of pregnancy when we diagnosed an acute pregnancy induced TTP. From then on until the 29th week she was treated with initially daily plasma exchanges (PE) followed by two weekly PE's. Thereafter until now she showed no relaps and the child was born by Caesarean section at the end of week 39.

Aims: We would like to show a case report.

Methods: ADAMTS13 activity, antigen and inhibitor (ELISA, Fa.Technoclone).

Results: Since week seven we overlook 37 complete sets of her ADAMTS13 properties (activity, antigen and inhibitor) determined with Technoclone kits. From week seven until week 29, when PE's were stopped, she showed a discrepancy between ADAMTS13-activity and antigen with much higher ADAMTS13 concentrations (mean 0.27 µg mL⁻¹, range 0.11 – 0.53 µg mL⁻¹, normal 0.5–1.6 µg mL⁻¹) than activities (mean 3.8%, range 1.8 – 7%, normal 50 – 110%). From week 30 till birth, her ADAMTS13 properties were perfectly normal with no discrepancies between activity and antigen. But astonishingly she shows since childbirth again a discrepant pattern with hardly detectable ADAMTS13 antigens (between 0.03 and 0.07 µg L⁻¹) but normal activities (57 – 92%) at all seven timepoints. Between 2009 and now we detected the pattern the patient showed after birthing in 22 additional patients (10 with acute TTP relapses, 3 with unrelated diseases and in the others we had no information). The relapsing patients had activities between 18 and 103% which would doubt the diagnosis of TTP, while the antigens were severely depressed (0.019 – 0.1 µg mL⁻¹).

Conclusion: Circulating immunocomplexes (IgG antibody and ADAMTS13) by acute TTP in pregnancy save the unborn child.

Disclosure of Interest: None declared.

PO042-WED

Anti-ADAMTS13 antibody features (IG isotype titers, functional ADAMTS13 inhibitor and circulating immune complexes [CIC] in patients with acquired thrombosis thrombocytopenic purpura (ATTP) at first consultation in our institution

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Background: aTTP form is often caused by circulating antibody (ab). The formation of CIC may be a pathophysiologic mechanism in aTTP.

Aims: To evaluate the relationship of ADAMTS13-measurements in 40 patients (P), 28 in acute phase (ap) and 12 non acute phase (nap).

Methods: ADAMTS13 activity (act), antigen (ag) and Free IgG anti-ADAMTS13 ab (free IgG ab) were evaluated by ELISA kits. Functional ADAMTS13 inhibitor (FI) was detected as Ferrari (2007) described. Detection of CIC by co-immunoprecipitation (WB) and ELISA with rabbit anti-ADAMTS13 (ABCAM). Informed consent and ethical approval were obtained.

Results: In ap, act was severely reduced in 26/28P (93%) and reduced in 2/28P (7%). Within 26P Ag was undetectable in 7 (27%), severely reduced in 3 (11%) and reduced in 14 (54%). FI was positive in 24/28P (86%). Free IgG ab was positive in 24/28P (86%). In nap, act was severely reduced in 3/12P (25%) and reduced in 6/12P (50%). Within those 12P, ag was severely reduced in 1 (8%) and reduced in 6 (50%). FI was positive in 8/11P (73%). Free IgG ab was positive in 10/12P (83%). Both Free IgM ab and IgA ab were positive in 1/12P (8%). The capturing ab for CIC showed good sensitivity when tested using a P with a CIC of cut-off of 1.93 normalized OD units. The nonparametric correlation $n = 9$, Spearman $r = 0.695$, $P < 0.04$ between CIC-ELISA and WB was significant ($\alpha=0.05$). In ap, CIC was detected in 9/28P, in 8/26P with severe act deficiency the prevalence of CIC was 33% (95% CI, 16–50%) and in 1/28P with reduced act. Within those 9P, the ag was undetectable in 2P, severely reduced in 2P and reduced in 4P; free IgG ab in 5P and positive FI in 8P. In naP, CIC was detected in 3/12P and in 2/6P with reduced act the prevalence of CIC was 40% (95% CI, 9–70%). Within those 3P ag was reduced in 2, free IgG ab in 2P and negative FI

Conclusion: Only one-third of P with aTTP displayed CIC. If the determination of circulating CIC in a first consultation is of value in predicting potential relapse will be further investigated increasing the number of P with aTTP.

Disclosure of Interest: None declared.

PO043-WED

ADAMTS13 gene expression is increased in kidney and liver in type 1 diabetes mellitus animal model

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Background: ADAMTS13 is a proteolytic enzyme responsible for large vWF multimers degradation, involved in blood clotting. Its proteolytic activity prevents the deposition of platelets along capillary lumina under the conditions of high shear stress in glomerular capillaries, commonly observed in diabetic nephropathy. This enzyme is primarily

synthesized in the liver, but is also expressed on platelets, endothelial cells and kidney and is normally present in plasma.

Aims: The aim of this study was to compare the ADAMTS13 gene expression in liver and kidney from type 1 diabetic and control (non-diabetic) mice.

Methods: Type 1 diabetes mellitus was induced in male C57BL/6J mice by intraperitoneal injection of 50 mg kg⁻¹ streptozotocin in citrate buffer (pH 4.5) for five days. Mice in the control group were injected with citrate buffer. All procedures were in accordance with the ethical standards of the Ethical Research Committee of the Universidade Federal de Minas Gerais (UFMG) and with the Helsinki Declaration. The mRNA was obtained from liver and kidney tissues and the ADAMTS13 gene expression was investigated by real-time PCR using a hydrolysis probe. Relative quantification was performed in relation to 18S rRNA gene expression by the 2^{-DeltaDeltaCt} analysis method.

Results: The liver showed higher level of ADAMTS13 gene expression when compared to kidney in both groups. Higher ADAMTS13 gene expression was detected in kidney (2.35×) from diabetic animals when compared to control. However, the difference in the ADAMTS13 gene expression in the liver was lower when compared the two groups (1.20×).

Conclusion: The results indicate that hepatic and renal ADAMTS13 gene expression in diabetic mice was higher when compared to control. Besides, the higher level of ADAMTS13 gene expression in kidney could be associated to a hypercoagulability compensatory mechanism observed in diabetic nephropathy.

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PO044-WED

An activity-based approach to anti-ADAMTS 13 antibodies

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Background: Antibodies against coagulation-related entities can be detected by immunological assays as well as activity-based assays. For example, antibodies associated with HIT II are diagnosed with both methods (HIPA test, anti-PF4 ELISA). On the other hand, coagulation factor inhibitors are usually detected in activity-based Bethesda assay while anti-ADAMTS 13 antibodies are detected using an ELISA.

Aims: An activity-based assay similar to the Bethesda method was performed to quantify anti-ADAMTS 13 antibodies. The method was compared to a classical inhibitor ELISA in patients with pathological ADAMTS 13 activity.

Methods: ADAMTS 13 parameters (activity, antigen and inhibitor) were analysed using test systems from Technoclone GmbH (Vienna, Austria). In cases of pathological activity the inhibitor was measured in the ELISA and, additionally, with a Bethesda-like method based on ADAMTS 13-activity measurement after incubation with normal plasma over 2 h.

Results: Discrepancies between immunological and activity-based assays were observed in regard to anti-ADAMTS 13 antibodies. Only 50% of the inhibitors measured in the ELISA method are positive in the Bethesda-like assay. The other half was found to be non-neutralizing and may be not all of them have diagnostic importance. There is no correlation between inhibitor titre in the ELISA and activity-based assay.

Conclusion: Inhibitors in ELISA and activity-based assays show significant discrepancies. Whether those are of diagnostic relevance remains an open question. Furthermore, the Bethesda-like method can be too insensitive to recognize inhibitors against ADAMTS 13. Similar to HIT II or FVIII inhibitors, anti-ADAMTS 13 inhibitors without

direct impact on ADAMTS 13 activity may overestimate the incidence of clinical relevant inhibitors. The design of an assay determines the results, which should be taken in consideration in clinical studies.

Disclosure of Interest: None declared.

PO045-WED

Detection of plasma ADAMTS13 activity using *E. coli* derived VWF-A2 substrates and microsphere-cytometry assays

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Background: Protease levels in human blood are often prognostic indicators of inflammatory, thrombotic or oncogenic disorders. The measurement of such enzyme activities in substrate-based assays is complicated due to the low prevalence of these enzymes and steric hindrance of the substrates by the more abundant blood proteins.

Aims: To address the above limitations, we developed a molecular construct that is suitable for microsphere-cytometer based assays in the milieu of human blood plasma. We demonstrate the utility of this substrate in measuring metalloprotease ADAMTS13 activity.

Methods: The substrate, expressed in *E. coli* as a fusion protein, contains the partial A2-domain of von Willebrand factor (VWF amino acids 1594–1670) that is modified to include a single primary amine at the N-terminus and free cysteines at the C-terminus. This design enabled N-terminus fluorescence conjugation using NHS (N-hydroxy-succinimide) chemistry. Maleimide-PEG (Polyethylene glycol)_n-biotin coupling at the C-terminus was performed with different lengths of PEG spacers.

Results: Once bound to streptavidin-bearing microspheres, the substrate fluorescence signal decreased in proportion with ADAMTS13 concentration. Whereas recombinant ADAMTS13 activity could be quantified using substrates with all PEG repeat-lengths, only the construct with the longer 77 PEG-unit could quantify proteolysis in blood plasma. Using this longer substrate, plasma ADAMTS13 levels as low as 5% could be detected within 30 min. Such measurements could also be readily performed under conditions resembling hyperbilirubinemia. Enzyme catalytic activity was tuned by varying buffer calcium, with lower divalent ion concentrations enhancing cleavage.

Conclusion: The study highlights the substrate design features important for microsphere-cytometry based proteolysis assays in the setting of human plasma.

Disclosure of Interest: None declared.

Animal Models III

PO046-WED

The effect of peptide-nootropics on platelet aggregation induced by epinephrine

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Background: It is known that stress has a significant effect on hemostasis system. However, these studies do not related to the final link of this reaction, namely, the influence of catecholamines in the blood cells in response to stress. Peptides nootropics thyroliberin (TRH) and analogue AKTG4-10 Semax, having antistressogenic action affect various parts of the blood coagulation system.

Aims: The aim of this work was to study the effects of TRH and semax on platelet aggregation under the influence of epinephrine.

Methods: Experiments were carried out on white rats in compliance with international principles of the Helsinki Declaration on the humane treatment of animals. In experiments *in vitro* to a sample with a suspension of washed platelets were added 0.85% NaCl or peptides in a range of concentrations and 10^{-3} – 10^{-10} M CaCl_2 (0.025 M). Platelet aggregation was recorded on a aggregometer Trombolite. Aggregation inhibition index was calculated.

Results: *In vitro* experiments studied the effect of TRH and semax on platelet aggregation under the action of epinephrine. It is shown that TRH, when added to a suspension of washed platelets in the whole range of concentrations induced inhibition of platelet aggregation, and adding TRH concentration of 10^{-3} – 10^{-4} and 10^{-7} M (inhibition index 26% – 42%, $P < 0.001$). Semax, unlike TRH, only at concentrations of 10^{-9} and 10^{-10} M. provided a small but statistically significant inhibition of epinephrine-induced platelet aggregation with inhibition index of 17.3% and 19.2%. Inhibition index TRH by this concentration was 25–30%. Thus, neuropeptide semax had a weaker effect on epinephrine-induced platelet aggregation than TRH.

Conclusion: Therefore, TRH and semax, reducing platelet aggregation, by the action of epinephrine, reduce the risk of thrombotic complications in stressor exposure, but TRH has a more power and pronounced effect.

Disclosure of Interest: None declared.

PO047-WED

Evaluation of the bleeding tendency in murine models of hemophilia and heparin therapy using the thrombodynamics assay

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Background: Estimation of the global coagulation in mice may expand the *in vivo* models but is challenged due to pre-activation of murine blood during collection, variation in the model conditions, and a large sample volume required for an assay.

Aims: To test an adequacy of the thrombodynamics assay for the hemostasis evaluation in murine models of hypocoagulation by comparison with the tail bleeding model.

Methods: Pre-anesthetized C57BL/6 and BALB/C mice were injected with 170 IU kg^{-1} bw unfractionated heparin (UFH) (heparin therapy model), 30 μg kg^{-1} anti-FVIII (hemophilia A model), or a saline (normal hemostasis) via the retrobulbar plexus. Then tail was cut (3 mm) and placed into warm saline; the bleeding time and blood loss were monitored for 20 min. The TD assay: blood (0.5 mL) was collected from the open heart in a syringe with citrate; the rate of clot growth (V) from immobilized human tissue factor was measured in un-diluted sample of platelet free plasma. FVIII level was measured with the clotting assay.

Results: (i) Hemostasis was repressed in antibody-induced hemophilic C57BL/6 mice (5% FVIII) as indicated by increased blood loss after tail tip amputation and by reduction in the clot growth rate *ex vivo* compared to the normal mice (see Table). Also the UFH treatment resulted in prolonged bleeding and suppression of the clot growth in the TD assay.

	Blood loss (μL)	V ($\mu\text{m min}^{-1}$)	FVIII (%)		Bleeding time (s)	V ($\mu\text{m min}^{-1}$)
'Hemophilia A' ($n = 6$)	353 $\pm$ 54*	3.7 $\pm$ 0.7*	5.4 $\pm$ 1.0*	'Heparin therapy' ($n = 6$)	941 $\pm$ 293	0.6 $\pm$ 0.1*
Control ($n = 8$)	16 $\pm$ 6	15.2 $\pm$ 0.6	80 $\pm$ 20	Control ($n = 9$)	71 $\pm$ 14	18.3 $\pm$ 1.3

* $P < 0.05$ compared to the control (Mann–Whitney test).

(ii) Inter-strain variation in response to UFH was found. Addition of UFH *in vitro* into plasma of C57BL/6 and BALB/C mice inhibited the clot growth rate with IC50 of 0.01 and 0.02 IU mL⁻¹, respectively. In human plasma IC50 was 0.1 IU mL⁻¹ according to the lower anti-thrombin activity in human.

Conclusion: Murine hemostasis may be adequately estimated with the TD assay that correlates with the bleeding tendency.

Disclosure of Interest: V. Kolyadko: None declared, S. Surov: None declared, S. Buvaliy: None declared, V. Kuznetsov: None declared, T. Vuimo: None declared, F. Ataullakhanov Shareholder of: HemaCore LLC that develops the thrombodynamics assay for use in the coagulation diagnostics, M. Pantelev Shareholder of: HemaCore LLC that develops the thrombodynamics assay for use in the coagulation diagnostics.

PO048-WED

Assessment of MOD-5014, a long acting FVIIA, pharmacokinetic, pharmacodynamics and correction of hemophilic coagulopathy in dogs with hemophilia A as part of the preparation for first in human study

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Background: OPKO (f.k.a PROLOR) is a clinical stage company developing long acting versions of therapeutic proteins utilizing CTP technology. This involves fusion of the C terminus peptide of hCG to the protein. The technology was clinically validated for several drugs while maintaining their biological activity.

Aims: The study aim was to characterize MOD-5014 PK, PD and correction of coagulopathy in hemophilia A dogs.

Methods: MOD-5014 was expressed in CHO cells, purified and activated utilizing specific purification process. MOD-5014 was assessed in hemophilic dogs in two phases. In the first phase the lowest dose of MOD-5014, which *in-vitro* improved TEG parameters, was considered the minimal effective dose. Based on the first phase the second phase was designed and doses were selected (50 -600 $\mu\text{g kg}^{-1}$ IV). During the second part, the *in-vivo* phase dogs were dosed twice over a 10 days period and the TEG parameters, MOD-5014 PK and PD, PT and aPTT as well as clinical were collected and analyzed.

Results: Infusions were well tolerated with no injection site reactions or significant changes in lab results. MOD-5014 demonstrated a dose dependent response as reflected by TEG, PK and PD parameters and normalized the response to maximal efficacy maintained for a long period of time. MOD-5014 performance was compared to published data for commercial rhFVIIa further confirming MOD-5014 long acting properties (3–4 fold higher compared to rhFVIIa)

Conclusion: In this study the PK, PD and coagulopathy in hemophilic dogs were further assessed. Attachments of CTP to FVIIa led to a markedly prolonged half-life, increased exposure (normalized AUC) and improved TEG, suggesting a prolonged hemostatic effect as compared to rFVIIa. Our data further confirm that CTP fused FVIIa is a safe, well tolerated and effective long acting FVIIa. Finally, the data

obtained in this study will assisted in establishing the dose range in the ongoing phase 2a study.

Disclosure of Interest: None declared.

PO049-WED

Safety evaluation of chronic antithrombin silencing in non-human primate and expanded therapeutic index in a hemophilia A mouse model

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Background: ALN-AT3, a subcutaneously administered investigational RNAi therapeutic targeting antithrombin (AT), is currently in clinical development (NCT02035605) for the treatment of hemophilia A and other rare bleeding disorders. It has previously been demonstrated that once weekly dosing of ALN-AT3 results in potent, dose-dependent and reversible silencing of plasma AT in multiple preclinical species.

Aims: The objective of these studies was to evaluate the tolerability of ALN-AT3 when administered over a chronic dosing period to normal (non-diseased) cynomolgus monkeys at therapeutically relevant dose levels and transgenic hemophilia A (HA) mice at exaggerated dose multiples.

Methods: ALN-AT3 was administered weekly to monkeys (6/sex/grp) for 39 wks at 0 (saline), 0.15, 0.3 and 0.5 mg kg⁻¹ (20, 50 and 60% steady state AT inhibition, respectively). ALN-AT3 was administered weekly to HA mice (35/sex/grp) for 26 wks at 0, 10 and 30 mg kg⁻¹ (20- and 60-fold multiples of the mouse ED50). Potential effects were evaluated by clinical signs, body weight, clinical pathology (hematology, chemistry, coagulation), plasma AT levels, organ weights, and histopathology.

Results: ALN-AT3 was well tolerated in monkeys with no adverse changes to clinical condition or clinical or anatomic pathology parameters. There were no significant alterations to coagulation parameters (clotting times, fibrinogen, d-dimers). ALN-AT3 was also well tolerated in HA mice. HA mice are inherently fragile and vulnerable to procedural stress, and as such there was a prominent incidence of early mortality in saline-treated control animals (40% overall). In contrast, mortality was significantly reduced in ALN-AT3-treated groups (6% overall).

Conclusion: Chronic administration of ALN-AT3 was well tolerated in monkeys, a relevant preclinical model. Chronic dosing at exaggerated dose levels (> 90% AT inhibition) in HA mice was also well tolerated and resulted in a survival benefit relative to saline-treated controls.

Disclosure of Interest: S. Barros Shareholder of: Alnylam Pharmaceuticals, Employee of: Alnylam Pharmaceuticals, K. Qian Shareholder of: Alnylam Pharmaceuticals, Employee of: Alnylam Pharmaceuticals, J. Qin Shareholder of: Alnylam Pharmaceuticals, Employee of: Alnylam Pharmaceuticals, B. Bettencourt Shareholder of: Alnylam Pharmaceuticals, Employee of: Alnylam Pharmaceuticals, A. Sehgal Shareholder of: Alnylam Pharmaceuticals, Employee of: Alnylam Pharmaceuticals, A. Akinc Shareholder of: Alnylam Pharmaceuticals, Employee of: Alnylam Pharmaceuticals.

PO050-WED

An antibody pretreatment strategy for improving cell-based therapy in a mouse model of hemophilia BHsu Y-C^{1,2}, Fan M-N¹, Huang Y-J³, Yang S-J¹, Yu I-S^{1,4}, Wu Y-M⁵ and Lin S-W^{1,2}¹Graduate Institute of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, National Taiwan University;²NTU Center of Genomic Medicine; ³Institute of Biotechnology, National Taiwan University; ⁴Laboratory Animal Center, College of Medicine, National Taiwan University; ⁵Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan

Background: Although factor IX (FIX) product replacement therapy for hemophilia B is quite successful, such therapy is a life-long treatment rather than a cure for the disease. Hepatocyte transplantation has been developed as a promising cell-based therapy for hemophilia B. However, the poor engraftment efficiency of donor cells usually limits its clinical applications. In previous study, we found that the engraftment efficiency of transplanted hepatocytes was increased in the recipient mice that had narrower hepatic sinusoids. Thus, we propose that narrow hepatic sinusoids that enhance the efficiency of hepatocyte engraftment may contribute to successful hemophilia B therapy by hepatocyte transplantation.

Aims: The aim of the study was to develop a strategy for successful hepatocyte transplantation.

Methods: To test the hypothesis, we injected syngeneic primary hepatocytes into hemophilia B mice. The hepatocytes that expressed high activity FIX protein by AAV-delivery of FIX gene into hemophilia B were used as donor cells. An antibody Ab-1E that causes narrow sinusoid width was used for preconditioning before transplantation.

Results: We observed that the amount of transplanted cells was nearly 2 times more in the liver of Ab-1E pretreated mice than those of IgG treated mice at all the time points analyzed for 1 month. We compared the therapeutic effect of higher transplantation efficiency by Ab-1E pretreatment in hemophilia B mice. Using AAV-FIX expressing donor hepatocytes for transplantation, we found that Ab-1E-pretreated hemophilia B mice had significantly higher antigen levels and FIX activity than the control groups that were pre-treated with IgG isotype control. Moreover, Ab-1E pretreated mice receiving functional FIX expressing hepatocytes also showed superior hemostatic protection and shortened tail-bleeding time, compared to control groups.

Conclusion: The Ab-1E antibody pretreatment strategy is effective and useful for improving the cell-based treatment strategy for hemophilia B in mice.

Disclosure of Interest: None declared.

PO051-WED

Effects of ankaferd blood stopper on cutaneous wound healing: experimental studyOzatli D¹, Kelahmetoglu O², Bozkurt A³, Tayfur V⁴, Bilge S³, Buyukkaya P¹, Yildiz L⁵ and Fisgin T⁶¹Hematology, Ondokuz Mayıs University, Medical Faculty, Atakum SAMSUN; ²Plastic Surgery, Tarsus State Hospital, İçel; ³Pharmacology; ⁴Plastic Surgery; ⁵Pathology, Ondokuz Mayıs University, Medical Faculty, Atakum SAMSUN; ⁶Hematology, Bahcesehir University, Istanbul, Turkey

Background: Ankaferd Blood Stopper® (ABS) offered as a hemostatic agent is a standardized herbal extract obtained from five different plants and it has been approved for the clinical management of external post-surgical and post-dental surgical bleedings in Turkey. ABS represents its hemostatic effect by promoting the rapid formation of a protein network covering the classical cascade model of the clotting system without independently acting on coagulation factors and platelets.

Aims: In the current study we tried to show effects of ABS on cutaneous wound healing.

Methods: In this study, full thickness wound model was created. Totally thirty six Sprague-Dowley rats were used as control group ($n:18$) and experimental group ($n:18$). After completing surgery, for control group 0.1 mL of saline solution was applied, while for experimental group 0.1 mL of ABS was applied. The wound dimensions were followed with taking photographs on forth, eighth and twelfth days. Histopathologic investigations including the count of inflammatory cells, fibroblast proliferation, value of collagen, vessel proliferation, re-epithelialization, ulcer, foreign body and necrosis (ischemia, fat) were evaluated with excised biopsies on those days.

Results: No significant differences in wound healing and histopathologic investigations were between control and experimental groups ($P > 0.05$).

Conclusion: Nor increasing or decreasing influence of ABS was showed on wound healing. It could be thought that ABS can be used safely during healing.

Disclosure of Interest: None declared.

PO052-WED

Assessment of knee joint bleeding in hemophilia B mice by fluorescence molecular tomographyChappell E¹, Sun J², Monahan PE², Appa RS³, Wiinberg B⁴, Hoffman M⁵ and Monroe DM¹¹McAllister Heart Institute; ²Gene Therapy Center, University of North Carolina at Chapel Hill, Chapel Hill, USA; ³Exploratory ADME; ⁴Translational Haemophilia Pharmacology, Novo Nordisk A/S, Malov, Denmark; ⁵Pathology, Duke University, Durham, USA

Background: One of the main morbidities in hemophilia is arthropathy caused by bleeding into joints. Exposure to blood and iron causes synovial thickening, cartilage erosion and the eventual fusion of joints, which can be extremely deleterious for hemophilia patients. However, joint bleeds are difficult to evaluate in the clinic. Several key aspects of joint bleeds, including how long they persist, the volume of blood that enters the joint and the blood clearance rate, are not understood and complicate effective treatment. In this study, fluorescent molecular tomography is coupled with an established mouse model of knee joint injury to describe hemophilic joint bleeds.

Aims: To detect and quantify knee joint bleeding and clearance in hemophilia B (FIX^{-/-}) mice.

Methods: FIX^{-/-} and wild-type mice were injected with a near-infrared vascular imaging probe (Angiosense, Perkin Elmer), a marker for blood flow, and visualized by fluorescence molecular tomography. After a knee joint injury, fluorescent signal can be detected as blood enters the intra-articular space and is cleared over time. Three-dimensional imaging permits quantification of the fluorescent signal in regions of interest within the mouse. Damage 2 weeks after injury was assessed through histology.

Results: FIX^{-/-} mice bleed into joints to a much greater extent than wild-type mice and are prone to rebleeds. The propensity of each mouse to clear the injected probe from the synovial space was evaluated.

Conclusion: Fluorescence molecular tomography provides a useful tool for measuring joint bleeding in a living mouse and can be used to investigate key parameters such as the initial rate of bleeding, the peak blood volume in the joint and the rate of clearance.

Disclosure of Interest: E. Chappell Grant/Research Support from: Novo Nordisk A/S, J. Sun Grant/Research Support from: Novo Nordisk A/S, P. Monahan Grant/Research Support from: Novo Nordisk A/S, R. Appa Employee of: Novo Nordisk A/S, B. Wiinberg Employee of: Novo Nordisk A/S, M. Hoffman Grant/Research Support from: Novo Nordisk A/S, D. Monroe Grant/Research Support from: Novo Nordisk A/S.

PO053-WED

Serum endocan levels as a potential non-invasive biomarker for diagnosing different hepatic diseasesPalumbo R¹, Uprichard S², Cotler S², Layden T², Fareed J¹ and Hoppensteadt D¹¹Pathology; ²Hepatology, Loyola University Medical Center, Maywood, USA

Background: Endocan, an endothelial proteoglycan, has been identified as a biomarker for hepatocellular carcinoma (HCC). HCC develops in the context of confounding hepatic diseases which is associated with thrombosis, inflammation and endothelium remodeling.

Aims: The aim of this study was to determine the endocan levels associated with liver disease and compare it with markers of inflammatory responses.

Methods: Endocan, interleukin 6 (IL6), tumor necrosis factor alpha (TNF-alpha), vascular endothelial growth factor (VEGF) and alpha-fetoprotein were quantified utilizing commercially available ELISA methods in plasma samples ($n = 20/\text{group}$) from patients with different liver conditions.

Results: The following table shows elevated levels of endocan and IL6 associated with not only HCC but also hepatitis C (HCV), autoimmune hepatitis (AIH), non-alcoholic steatohepatitis (NASH), and alcoholic liver disease (OHL). Endocan was elevated to a similar extent in HCC compared to other liver diseases. Endocan and IL6 were found to be significantly elevated in NASH relative to non-alcoholic fatty liver disease (NAFLD), two disease states which are difficult to discriminate without a biopsy.

PO054-WED

Effect of a recombinant plant elastase inhibitor with anti-inflammatory action – RBBCI (bauhinia bauhinioides cruzain/cruzipain inhibitor) on a rat model of venous thrombosisOliva ML¹, Oliveira C¹, Valois MV¹, Ottaiano T¹, Lima M¹, Miranda A¹, Sampaio M¹ and Maffei FH²¹Universidade Federal De São Paulo; ²Departament of Surgery and Orthopedics, UNESP, São Paulo, Brazil

Background: We had demonstrated previously the anti-inflammatory effect of a serine and cysteine peptidase inhibitor BbCI that blocks elastase, cathepsins G and L and proteinase-3. The recombinant rBbCI, expressed in *E. coli*, shows the same blocking activity. Heparin, besides its anticoagulant effect, has similar inhibitory properties by inhibiting elastase and cathepsin G.

Aims: In the present study, we investigated the effect of rBbCI comprising histidine-tail in an experimental venous thrombosis model comparing to that of heparin effects.

Methods: Wistar rats were anaesthetized, and randomly allocated into 5 groups: rBbCI (0.45 mg kg⁻¹), rBbCI (1.40 mg kg⁻¹), unfractionated heparin (UFH) (50 IU kg⁻¹), UFH (100 IU kg⁻¹) and sodium chloride (control). Twenty minutes after drug administration, thrombosis was induced by ligation of the vena cava (VC) and its branches. Four hours later, the animals were re-anesthetized, the vein dissected free and opened. Thrombus, if present, was withdrawn and weighted. Activated partial thromboplastin time (aPTT) and Prothrombin time (TP) were measured and TNF- α , GRO/KC, MCP-1, MIP-1A, IL-1 α , IL-1 β and IL-6 determined, at the end of the experiment, in blood taken by cardiac puncture. The animals were killed by an overdose of anesthetics. aPTT and bleeding time was measured in a different group of animals submitted to a sham operation at the time of VC ligation.

Results: A significant decrease in the thrombus weight in relation to the control group was verified in the heparin 100 IU kg⁻¹ and rBbCI 1.40 mg kg⁻¹ groups without difference between these two groups. The other groups did not differ from control. No differences in coagulation parameters were detected among groups at the end of experiment but, the GRO/KC was reduced in the rBbCI 1.40 mg kg⁻¹ group. In the sham operation groups aPTT was prolonged only in the heparin group.

Conclusion: In conclusion, in this model, rBbCI showed an antithrombotic property without anticoagulant effect.

Disclosure of Interest: None declared.

Disease	Endocan (ng mL ⁻¹)	IL 6 (pg mL ⁻¹)	TNF alpha (pg mL ⁻¹)	VEGF (pg mL ⁻¹)	Alpha –fetoprotein (ng mL ⁻¹)
HCC	7.30 (11.00)	7.45 (8.40)	1.25 (3.10)	23.27 (108.00)	12.62 (19.20)
HBV	1.48 (3.80)	0.00 (3.10)	0.74 (1.00)	118.89 (143.10)	2.88 (1.60)
HCV	5.26 (9.30)	2.53 (5.10)	1.52 (5.40)	51.13 (48.70)	4.70 (5.10)
AIH	5.02 (8.00)	3.09 (4.70)	0.00 (2.20)	86.83 (107.50)	3.40 (3.00)
NASH	5.27 (6.80)	6.99 (19.40)	1.13 (1.20)	46.43 (54.00)	2.95 (2.80)
NAFLD	2.15 (4.20)	0.00 (2.80)	1.53 (1.90)	42.21 (76.80)	3.59 (2.10)
OHL	6.29 (8.60)	8.09 (17.80)	1.22 (2.90)	43.75 (75.00)	3.54 (3.30)
Normal controls	2.03 (3.90)	0.00 (2.60)	0.56 (0.30)	38.64 (29.60)	2.33 (2.40)

Conclusion: In conclusion, endocan may be useful as a non-invasive biomarker for liver cirrhosis and/or the clinical differentiation of liver diseases and the understanding of their pathogenesis.

Disclosure of Interest: None declared.

Antiphospholipid Syndrome II

PO055-WED

Evaluation of the importance of mixing patient plasma with a pool of normal plasmas when diagnosing lupus anticoagulant

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Background: According to international guidelines, three stages algorithm is required to confirm the presence of lupus anticoagulant: Detection of lengthening of time of one of the recommended coagulation tests, mixture with a pool of normal plasmas to detect whether it is due or not to an inhibitor, confirmation of the kind of inhibitor, antiphospholipid antibodies or not.

Aims: Evaluation of the importance of mixing patient plasma with a pool of normal plasmas when diagnosing lupus anticoagulant.

Methods: Sixty patients under clinical suspicion of lupus anticoagulant and elongation of clotting time, with at least one of two reagents: diluted Russell viper venom or Silica Clotting Time. Patients plasmas were mixed with a pool of normal plasmas and Rosner index was calculated to determine whether there was correction or not. Regardless the Rosner index result, confirmatory tests for each of the tests were performed. The conclusion about the presence or not of lupus anticoagulant based on the three traditional stages and the conclusion based on the confirmatory tests regardless mixtures results, were compared. All tests were performed with IL ACL TOP.

Results: Forty-five patients were positive and 15 patients were negative for lupus anticoagulant, when performing confirmatory tests without considering mixtures results, 13 of the positive ones, would not have been diagnosed as positive if we had relied on the study of mixtures to decide to confirm or not, and 9 of the negative ones would have been considered negative without confirmatory tests if we had relied on the study of mixtures to decide to confirm or not. **Conclusion:** The second stage, consisting of mixing studies, when investigating the presence of lupus anticoagulant, may lead to decide not to continue with confirmation tests, in patients who might result positive if the confirmation tests were performed. So sensitivity would be better if confirmation studies were always included, regardless mixing studies results.

Disclosure of Interest: None declared.

PO056-WED

The taipan snake venom time could be a reliable assay for the detection of lupus anticoagulant in patient receiving rivaroxaban

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Background: Anticoagulant treatment by oral anti Xa rivaroxaban compromises the biological detection of lupus anticoagulant (LA) with assays such as dRVVT, with frequent false positive results. Recently, the Taipan snake venom time (TSVT) coupled with Ecarin time (ET) has been proposed for detecting LA in patients receiving rivaroxaban, because both venoms are direct prothrombin activators and unaffected by FXa inhibitors.

Aims: The aim of our study was to evaluate the performances of TSVT for the detection of LA in patients receiving or not rivaroxaban.

Methods: Patients and methods: plasma samples from 30 healthy subjects, 15 patients with antiphospholipid syndrome (APS) and positive dRVVT (group LA+) and 15 patients with thromboembolism without

LA (group LA-) were spiked with rivaroxaban (50 to 500 ng mL⁻¹). dRVVT and TSVT were performed in the presence or the absence of rivaroxaban. Furthermore, plasma samples from 19 patients treated by rivaroxaban for thrombosis, were also tested.

Results: We first confirmed that frequent false positive results of dRVVT screen were obtained (90%), with a false positive 'dRVVT confirm' in about half of samples containing the lower concentration of rivaroxaban (50 ng mL⁻¹). In contrast, TSVT was not modified by rivaroxaban since TSVT ratio remained < 1.13 in plasma samples from healthy and 'LA-' subjects spiked or not with rivaroxaban ($P = 0.1$). When 'LA +' samples were tested, a TSVT ratio > 1.13 was obtained in 11 cases/15 (sensitivity 73.3%) and the specificity of the assay was 93.3% due to one false positive result. In addition, in our hands, ET did not increase the performances of TSVT. In addition, two positive LA samples were detected with TSVT among the 19 patients treated with rivaroxaban, and the diagnosis of APS was further confirmed in one of them.

Conclusion: The TSVT can be useful to detect LA in patients treated with rivaroxaban. However, this assay requires in house development and validation in larger cohorts of patients.

Disclosure of Interest: None declared.

PO057-WED

New mixing test index to discriminate between lupus anticoagulant and coagulation inhibitor

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Background: Mixing test is used to discriminate between inhibitors and factor deficiency, and is recommended for lupus anticoagulant (LA) and coagulation inhibitor detection. However, there has been little study to differentiate between LA and coagulation inhibitor.

Aims: To differentiate LA, coagulation inhibitor, factor deficient and heparin samples in activated partial thromboplastin time (APTT) mixing test.

Methods: We used 27 LA-positive, 29 factor deficient such as FV, VIII, IX and XI, 24 heparin and 48 coagulation inhibitor samples. The result is shown by the index of circulating anticoagulant (ICA): ICA = [(b - c) / a] X 100, where a, b and c are the clotting times of the patient, the 1:1 mixture, and normal plasma, respectively. Mixing tests were performed without incubation and after 2 h incubation at 37 degrees. ICA calculated by the clotting times without incubation and after 2 h incubation were defined as ICAi and ICA_d, respectively. ICA_d / ICAi and ICA_d - ICAi were also calculated, and the sensitivity and specificity were compared with those of ICAi and ICA_d. The cut-off values were established by ROC analysis. The study was approved by the Research Ethics Committee, and informed consent was obtained from all patients.

Results: The values of ICA_d / ICAi and ICA_d - ICAi in coagulation inhibitor were significantly higher than those of the other samples. The sensitivity for coagulation inhibitor of ICAi, ICA_d, ICA_d / ICAi and ICA_d - ICAi were 87.5%, 81.3%, 93.8% and 91.7%, respectively. The specificity were 45.0%, 66.3%, 85.0% and 98.8%, respectively. ICAi and ICA_d couldn't separate heparin and LA samples from coagulation inhibitors, although factor deficient samples showed low values in both indexes.

Conclusion: ICA_d / ICAi and ICA_d - ICAi showed significant differences between LA and coagulation inhibitor, and high sensitivity and specificity. These indexes can discriminate LA and coagulation inhibitor beside factor deficient and heparin samples.

Disclosure of Interest: O. Kumano Employee of: Sysmex, M. Ieko Grant/Research Support from: Sysmex, S. Naito: None declared, M. Yoshida: None declared, N. Takahashi: None declared, T. Suzuki Employee of: Sysmex, T. Aoki: None declared.

PO058-WED

Predictive markers for thrombotic events in autoimmune diseases: comparison of antiphospholipid score (APL-S) and global anti-phospholipid syndrome score (GAPSS)

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Background: Antiphospholipid score (aPLS) and Global Antiphospholipid Syndrome Score (GAPSS) are the scoring systems to quantify the probability for diagnosis of antiphospholipid syndrome (APS). These scores, as well as tools for diagnosis, function as indexes for predicting future thrombosis in autoimmune diseases.

Aims: To evaluate the diagnostic and predictive power of aPLS and GAPSS.

Methods: This study comprised 257 patients with autoimmune diseases including 42 APS who visited Hokkaido University Rheumatology Clinic between 2002 to 2003 with follow-up period of more than 5 years (median 126 (IQR 92.5, 133) months). Clinical data were obtained from medical charts. Five lupus anticoagulant assays and six ELISAs (IgG/M of anticardiolipin antibodies, anti- β 2-glycoprotein I antibodies and phosphatidylserine dependent antiprothrombin antibodies) were performed. To evaluate the diagnostic powers for aPLS and GAPSS, area under the curve (AUC) of receiver operating characteristic (ROC) curves were calculated. To evaluate the powers of two scores for thrombosis prediction, Cox proportional hazard regression analyses were performed with the cut off derived from ROC curve. Each risk factor for the multivariate Cox analyses were assessed through univariate regression test. To compare the predictive powers of the two scores, Somer's d coefficient was calculated.

Results: Thirty-seven patients newly developed thrombosis during the follow-up periods. The AUC of the ROC curve of two scores did not show significant difference. The Cox multivariate proportional hazard regression analyses revealed that both scores, with appropriate cut off levels, accurately reflected the risks of the future thrombosis (aPLS > 31 $P < 0.0001$, GAPSS > 10 $P = 0.01$). The aPLS showed higher Somer's coefficient D than GAPSS (0.497 vs. 0.410).

Conclusion: The aPLS and GAPSS accurately reflected the diagnosis of APS and the risk for future thrombotic events in patients with autoimmune diseases. APLS may have superiority on predicting future thrombosis compared with GAPSS.

Disclosure of Interest: None declared.

PO059-WED

Detection of antiphospholipid antibodies in normal pregnant women and in pregnant women at the time of preeclampsia onset

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Background: Preeclampsia (PE) is a systemic complication of pregnancy. Antiphospholipid Syndrome (APS) is an autoimmune disorder. PE is one of the obstetric criteria of the APS but the association between antiphospholipid antibodies (aPL) remains unclear and controversial. Identification of women at risk of PE can help us to indicate close monitoring and prophylactic treatment to minimize fetomaternal adverse outcome, whether aPL antibodies could be a possible biological marker of PE need to be established.

Aims: To determine whether aPL are present at the time of PE onset in comparison to normal pregnant women of more of

20 weeks of gestation. Design: Observational, prospective and analytic. Approved by the medical ethics committee. Informed consents were obtained.

Methods: Normal pregnant women (NPW) ($n = 40$): > 20 weeks of gestation without hypertension, antithrombotics and/or other pregnancy complications, delivering a newborn at term with adequate weight for gestational age. Patients: PE ($n = 100$); 73 with primary PE (PP) and 27 with superimposed SPE (27). Laboratory tests: Lupus anticoagulant (LA) according to the ISTH recommendations; ELISA tests aPL IgG and IgM (Louisville) anti β 2 Glycoprotein I antibodies IgG and IgM (ab2GPI). Blood samples were taken at the time of PE onset and in NPW after 20 weeks of gestation.

Results: The prevalence of LA in PE was 24% (24/100) in comparison with NPW 5% (2/40) ($P < 0.01$); when considering PP 27, 4% (20/73) and SPE 14, 8% (4/27) LA was only statistically significant in primary preeclampsia ($P < 0.01$). Only ab2GPI IgM levels were higher in PE (5.57 ± 3.21 U mL⁻¹ vs. 7.77 ± 4.57 U mL⁻¹ $P < 0.01$). Most of them in 'gray' area according to Louisville ELISA test.

Conclusion: In our study we found that the LA was the most important aPL test detected in women at the time of PE onset, It was only statistically significant in women with primary preeclampsia. Larger studies are needed to confirm the usefulness of aPL, in particular LA, as a PE risk marker; actually there is an absence of LA testing in many studies.

Disclosure of Interest: None declared.

PO060-WED

High avidity auto-antibodies against tissue factor pathway inhibitor are frequently observed in thrombotic antiphospholipid syndrome patients

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Background: Anti-tissue factor pathway inhibitor antibodies (α TFPI) have been reported in antiphospholipid syndrome (APS); which may impair TFPI function, thus contributing to hypercoagulability.

Aims: To investigate the frequency, avidity and effects of α TFPI in thrombotic APS.

Methods: 50 APS (28 with venous thromboembolism (VTE), 14 with arterial thromboembolism (AT), 8 with VTE and AT) and 50 non-APS patients (40 with VTE, 7 with VTE and AT) receiving warfarin for secondary thromboprophylaxis, and 75 normal controls (NC) were studied. 18/50 APS patients had a severe thrombotic phenotype (VTE as well as AT or recurrent thrombosis despite therapeutic anticoagulation). IgG α TFPI was measured by in-house ELISA using recombinant human TFPI (normal cut-off 61 U mL⁻¹) and avidity was determined using chaotropic conditions with increasing NaCl concentration. TFPI activity was measured by FXa inhibition and amidolytic assay (NR 79–129%). Thrombin generation (TG) was assessed using a 5 pM tissue factor trigger.

Results: α TFPI were seen in 20/50 APS (40%) and 9/50 (18%) non-APS patients ($P = 0.004$) and a trend towards higher α TFPI values was observed in APS (range: 62–213 U mL⁻¹) compared to non-APS (71–167 U mL⁻¹) patients. 12 APS and 3 non-APS patients had decreased TFPI activity levels, range: 46–75% and 61–75%, respectively. In the APS patients, TFPI activity was inversely correlated with α TFPI ($r_s = -0.4$, $P = 0.01$). High avidity IgG α TFPI was detected in 10 APS (all triple aPL positive) and one non-APS patients, and associated with decreased TFPI activity ($P = 0.03$); 8/10 APS and the single non-APS patient had a severe thrombotic phenotype. TG testing revealed 4 APS patients with increased peak thrombin and α TFPI (3 with high avidity); 3 of these had decreased TFPI activity. Decreased TFPI activity was also associated with a shorter TG lag time ($P = 0.03$).

Conclusion: Our results suggest that high avidity α TFPI contributes to the hypercoagulable state observed in thrombotic APS patients.

Disclosure of Interest: None declared.

PO061-WED

Pregnancy in women with antiphospholipid syndrome

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Background: Women with antiphospholipid syndrome (APS) are in increased risk for pregnancy complications including recurrent foetal loss (FL), intrauterine growth restriction (IUGR) or premature delivery. Treatment with low molecular weight heparin (LMWH) and aspirin improved the pregnancy outcomes, however, the management of pregnancy in APS still remains to be a major challenge.

Aims: To evaluate pregnancy outcomes in women with fully developed APS.

Methods: We managed 5 pregnancies in 4 women (age 25–37 yrs) with APS (3 primary and 1 secondary APS). All pats received aspirin 100 mg day⁻¹ since preconception plus full dose LMWH after the confirmation of pregnancy. Anti Xa activity was monitored monthly and ultrasound was performed in week 8, 20, 24 and later on monthly.

Results: Lupus anticoagulant, ACLA and anti-beta2GPI were pos-ve in all patients, 2/4 had a history of deep vein thrombosis (DVT)/ pulmonary embolism and 3/4 foetal loss. Pat 1 developed HELLP syndrome in week 34 (W34), pregnancy was terminated by cesarean section (CS) with a live birth. The newborn body weight (NBW) was 2200 g. In pat 2 expecting gemini moderate thrombocytopenia, hepatopathy and IUGR with oligohydramnion were diagnosed at W31. In a W32 cesarean section was performed, the BW of live newborns were 1365 g and 1300 g. Pat 3 intolerant to aspirin suffered from hepatopathy and severe thrombocytopenia since W14. CS was performed in a week 25 due to pre-eclampsia. A newborn with a BW of 500 g died 2 h after his birth. Pat 4 experienced four still births before the diagnosis of APS. LMWH plus aspirin in two consecutive pregnancies resulted in two premature live births, both in W28 by CS, with a NBW of 1300 g and 1250 g. She developed DVT in puerperium despite continuing thromboprophylaxis with LMWH.

Conclusion: We demonstrated that LMWH plus aspirin in pregnant women with APS improve the chance of live birth, however, other complications of placental dysfunction are still not fully avoided.

Disclosure of Interest: None declared.

PO062-WED

Antiphospholipid antibodies in cerebral vascular events

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Background: It has been suggested that antiphospholipid antibodies (APA) were associated to cerebral vascular events, especially in young patients with or without cardiovascular risk factors.

Aims: The aim of this study was to evaluate the risk of cerebral vascular events in young Tunisian population associated to APA.

Methods: This study included 82 young patients aged < 60 years old with cerebral events (ischemic stroke and cerebral venous thrombosis)

and 25 healthy controls matched with sex and age. Patients and controls were screened for APA: anticardiolipin / antiB2 glycoprotein antibodies with home reagents and lupus anticoagulant with DRVVT and A-PTT reagents from STAGO according to ISTH guidelines.

Results: There were 22 males and 60 females. The mean age was 39.7 years old [21–60]. Among the 82 patients, there were 53 with ischemic stroke and 28 with cerebral venous thrombosis. APA frequency was 7% in patients ($n = 6$) vs. 16% in controls ($n = 4$); $P = 0.24$. No patient with cerebral venous thrombosis had APA. Whereas, 5 patients with ischemic stroke were positive for APA screening: Lupus anticoagulant in one patient, Anti B2 glycoprotein IgM isotype in 2 patients and 2 controls, Anti B2 glycoprotein IgM and IgG isotypes in one patient and two controls and Anti B2 glycoprotein IgM associated to anticardiolipin IgM in one patient. Among the 6 patients with APA, one patient had recurrent transient ischemic attack and one patient had cardiovascular events.

Conclusion: These data demonstrated that APA in young patients with cerebral vascular events were not as frequent as reported in the literature. This could be a particularity in Tunisian population.

Disclosure of Interest: None declared.

PO063-WED

Antiphospholipid antibodies and the risk of stroke in Tanzania: results from a community-based case-control study

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Background: Stroke incidence is high in sub-Saharan Africa, which cannot be explained by the presence of traditional risk factors only. There is epidemiologic evidence that infections are associated with an increased risk of stroke. Antiphospholipid antibodies (aPL), which are thought to develop as a consequence of infectious diseases, might be one of the etiologic factors.

Aims: To analyse the contribution of aPL to the risk of stroke in a large community-based case-control study in Tanzania.

Methods: aPL profiles were determined in 158 patients with stroke, as well as 368 age- and sex-matched healthy controls recruited in both rural and urban settings in Tanzania.

Results: Out of the aPL subtypes often observed after infections, only the presence of anticardiolipin IgG was associated with stroke (Odds ratio (OR): 2.1, 95% confidence interval (CI): 1.0–4.3). This association disappeared when corrected for the presence of antibodies against *Treponema pallidum*. The presence of anti- β 2-Glycoprotein I (anti- β 2GPI) or anti-prothrombin antibodies was not associated with an increased risk of stroke. The prevalence of anti- β 2GPI antibodies in the Tanzanian healthy population was very high when Dutch cut-off values were applied (67%). The presence of lupus anticoagulant (LA) was associated with a 21-fold increased risk of stroke (CI: 7.2–60.5). Correction for hypertension, diabetes and syphilis did not affect the risk of stroke in patients with LA. The presence of LA was associated with a 48-fold increased risk of stroke (CI 6.3–375) in patients younger than 65 and a 12-fold increased risk of stroke (CI: 3.2–44.2) in patients older than 65.

Conclusion: The presence of LA is a strong risk factor for stroke in Tanzania. The risk of stroke associated with LA is higher in young and middle aged patients than in elderly patients.

Disclosure of Interest: None declared.

PO064-WED

Antiphospholipid syndrome alliance for clinical trials and international networking (APS action) core laboratory validation exercise: an international performance assessment of lupus anticoagulant (LA) tests using standardised methods and reagents

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Background: APS ACTION International Clinical Database and Repository is studying the natural course of disease over 10 years in persistently aPL-positive patients with/without other systemic autoimmune diseases. The protocol involves testing for LA at 5 international core laboratories.

Aims: To evaluate the performance of LA tests, in the 5 participating Core laboratories (CL, UK, Italy, USA, Australia & Brazil; anonymised to A-E in no particular order), using the same methodology.

Methods: All laboratories used an ACL TOP500 and the same reagent lot (DRVVT Screen/Confirm; silica clotting time (SCT) (Instrumentation Laboratory, Barcelona). Fresh vials of 1st International Reference Panel for LA (NIBSC) comprising LA negative (NLA), moderate positive (MLA) and strong positive (SLA) LA samples, as well as commercial QC were tested on each of 3 working days. Results were normalised with PNP.

Results: Within laboratory precision was good in four CL (cv 0.5–5%), but higher in the 5th CL (cv 2–7%), values increasing with LA potency). Similar DRVVT results were obtained between CL for normal QC and NLA plasma. However, for abnormal QC, MLA & SLA plasmas, one other CL tended to show longer clotting times (by up to 22 s) and higher LA ratios. In mixing studies (1:1 with PNP) there was a shortening of the clotting time for MLA & SLA plasmas, but they remained LA positive except for one laboratory with MLA. Four CL performed SCT and showed good agreement, although one CL tended to have shorter clotting times (approximately 8 s) in LA positive samples, but Screen/Confirm ratios compensated for this. The differences in clotting times and ratios could be due to sample preparation and handling, or the exact analyser protocol. One CL also tested the same reagents on a different analyser (CS-5100, Sysmex) and achieved similar LA ratios.

Conclusion: Despite the standardisation of samples, reagents and analysers, some local differences were observed. However, all laboratories correctly identified LA in the Reference Panel samples.

Disclosure of Interest: None declared.

PO065-WED

Beneficial effect of pravastatin in four pregnant women with antiphospholipid syndrome and ischaemic placental disease

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Background: Treatment of pregnant women with antiphospholipid syndrome (APS) focuses on anticoagulation therapy, mostly given empirically, and often ineffective. Ischaemic placental disease (IPD), including preeclampsia (PE) and intrauterine growth restriction (IUGR) is frequently associated with APS. Studies in animals suggest that pravastatin may protect pregnancies in APS.

Aims: We previously reported the clinical improvement and successful pregnancy in an APS patient who developed early PE leading to stillbirth during her 1st pregnancy and recurrent early PE in her 2nd pregnancy besides the prophylactic treatment with low molecular weight heparin (LMWH) and aspirin, treated with pravastatin 20 mg -P20. 15 days later preeclampsia disappeared and she delivered full term by normal delivery. Here we aimed to evaluate the effect of pravastatin in 3 more APS patients with IPD that did not respond to enoxaparin and aspirin (LMWH+ASP).

Methods: All 4 APS pregnant women were in prophylactic treatment with LMWH+ASP when IPD developed and received P20 (off label use) afterwards.

Results: Patient 2 conceived twins after 15th IVF- 2 miscarriages. At week 22 she developed PE. One foetus showed severe IUGR and abnormal umbilical artery Doppler. Termination of the pregnancy was recommended. She was given P20. Doppler measurements improved and foetal weight increased. Proteinuria decreased to 290 mg per 24 h and BP was normal. Both neonates were born alive at week 34.

Patient 3 developed HELLP syndrome leading to a stillbirth in 1st pregnancy. During 2nd pregnancy she developed severe IUGR. After P20 foetus weight increased and a healthy neonate was born at week 36.

Patient 4 has a history of miscarriages. Severe IUGR was observed at week 29 and she started on P20. 15 days later the foetus gained a significant amount of weight. A healthy baby (2780 g) was born at week 37.

Conclusion: These case reports suggest that pravastatin is a good treatment to prevent ischemic placental disease in women with APS.

Disclosure of Interest: None declared.

PO066-WED

Increased levels of tissue factor in antiphospholipid syndrome are associated with the diagnosis of systemic lupus erythematosus and may have no impact on disease prognosis

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Background: Tissue factor (TF) has been described as an important element of the pathogenesis of thrombosis caused by antiphospholipid

syndrome (APS). However, it is not clear if TF has impact on APS clinical course

Aims: To determine the circulating levels of TF in a cohort of patients with APS and previous thrombosis and to correlate these levels with APS-associated clinical presentation.

Methods: Plasma tissue factor was quantified by ELISA commercial kit. Clinical and laboratory data were collected retrospectively from medical records and direct questionnaires.

Results: Ninety-nine patients were included, 76% were female and 37% had systemic lupus erythematosus (SLE). Median age at time of the first thrombosis was 27 years old (IQ 20–39), median time since diagnosis was 7 years (IQ 3–13). Plasma levels of TF were higher in SLE-associated APS than in primary APS and healthy controls (medians: 306.6 vs. 149.9 vs. 149.2 pg mL⁻¹, respectively, $P < 0.001$). TF levels were not associated neither with recurrence, type of thrombosis (arterial or venous), obstetric complications nor with the profile of antiphospholipid antibodies. Time since diagnosis was not correlated with TF levels. TF levels were related with LDL ($r^2 = 0.10$, $P = 0.009$) and HDL cholesterol levels ($r^2 = 0.11$, $P = 0.007$), platelet counts ($r^2 = 0.05$, $P = 0.03$), lymphocyte counts ($r^2 = 0.05$, $P = 0.04$), and complement C3 levels ($r^2 = 0.09$, $P = 0.007$). Inflammatory markers, such as reactive C-protein, tumor necrosis factor alpha, interleukines-6 and -8 were not correlated with TF. In multiple linear regression, LDL, HDL cholesterol levels and platelet counts were the independent factors related to TF levels ($r^2 = 0.3$, $P < 0.001$).

Conclusion: TF may be increased in APS patients, particularly in those with SLE. In APS patients, cholesterol levels and platelet were associated with plasma levels of TF. However, TF levels were not associated with any APS complication. Thus, although being increased, TF plasma levels may not be a relevant marker of APS prognosis.

Disclosure of Interest: None declared.

PO067-WED

Distinct risk factors for arterial and venous thrombosis in antiphospholipid syndrome

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Background: Antiphospholipid syndrome (APS) is a pro-thrombotic disease that may present with a wide range of thrombotic complications. Risk factors associated with different thrombotic complications are not fully elucidated.

Aims: The aim of this study was to evaluate the clinical features of arterial and venous thrombosis in APS and identify potentially modifiable risk factors.

Methods: We performed a comprehensive evaluation of demographic, clinical and laboratory features of a retrospective cohort of patients with APS and previous thrombosis. Data were collected from medical records and specific questionnaires.

Results: One hundred patients were included in the study. VT occurred in 69% and AT in 31% of patients; 63% of patients had idiopathic APS. Patients with VT had lower HDL cholesterol levels (52 vs. 54 mg dL⁻¹, $P = 0.01$); higher frequency of tobacco use (36.8 vs. 12.5%, $P = 0.03$) and higher frequency of positive IgG- anticardiolipin antibody (60.3 vs. 29.6%, $P = 0.01$) than patients with AT. In counterpart, patients with AT tended to have higher frequency of systemic hypertension (55 vs. 32%, $P = 0.07$), lower hemoglobin levels (13.0 vs. 13.9, $P = 0.02$) higher platelet counts (251.000 vs. 203.500, $P = 0.02$), higher C-reactive protein levels (0.25 vs. 0.10 mg L⁻¹, $P = 0.05$),

higher frequency of positive lupus anticoagulant antibody (88 vs. 68%, $P = 0.05$) and higher frequency of obstetric morbidity (91.7 vs. 44%, $P = 0.01$), than VT patients.

Conclusion: Our results provide evidences that risk factors associated with AT and VT in APS are distinct. The occurrence of VT was associated with lipid disorders and smoking, while AT was associated with systemic hypertension and inflammation. Moreover, patients with arterial thrombosis also presented higher frequency of adverse pregnancy outcomes, which may suggest that arterial events are related to severe forms of APS.

Disclosure of Interest: None declared.

PO068-WED

Clinical relevance of the global antiphospholipid syndrome score (GAPSS) in differentiating between transient ischemic attack and stroke in patients with antiphospholipid antibodies

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Background: Arterial event are frequent in the antiphospholipid syndrome (APS) and stroke is a major clinical manifestation of syndrome.

Aims: In this study, we aimed to evaluate the clinical usefulness of the Global APS Score (GAPSS) in differentiating between transient ischemic attack (TIA) and stroke in a cohort of patients tested positive for aPL who suffered cerebrovascular events.

Methods: We included 40 consecutive SLE patients, all with a history of cerebrovascular events. Demographic, clinical and laboratory characteristics were collected. aPL profile included anticardiolipin antibodies (aCL), lupus anticoagulant (LA), anti β_2 glycoprotein I antibody (anti- β_2 GPI), and antibodies to phosphatidylserine-prothrombin complex (aPS/PT). Cardiovascular risk factors were assessed following NICE guidelines. The GAPSS system was calculated for each patient.

Results: Nineteen patients (47.5%) had stroke, 16 (40%) a transient ischemic attack (TIA), defined as neurologic symptoms or signs lasting < 24 h by a neurologist. Five patients (12.5%) experienced both. Thirty patients (75%) fulfilled the current APS classification criteria. Higher values of GAPSS were seen in patients who experienced stroke (alone or in association with TIA) when compared to those with TIA alone (10.13 ± 3.3 [range 5–16] vs. 6.3 ± 4.6 [range 3–13], $P = 0.033$). This observation was confirmed when compared patients who had a history of stroke alone with patients with TIA alone (9.95 ± 3.29 [range 5–16] vs. 6.3 ± 4.6 [range 3–13], $P = 0.029$). Patients who experienced both stroke and TIA showed the highest GAPSS but the difference was only statistically significant when compared to those with TIA alone (10.8 ± 3.63 [range 7–16] vs. 6.3 ± 4.6 [range 3–13], $P = 0.03$).

Conclusion: The GAPSS is a valid tool for risk stratification for ischemic manifestation in the setting of cerebrovascular events. This tool may help identifying a more 'at risk' population in whom a tailored prophylaxis therapy might be beneficial.

Disclosure of Interest: None declared.

PO069-WED

Evaluation of B2-glycoprotein I levels in patients with antiphospholipid syndrome

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Background: Antiphospholipid syndrome (APS) is characterized by the presence of antiphospholipid antibodies (aPL) associated with clinical manifestations (thrombosis and/or pregnancy complications). These manifestations are most strongly associated with the presence of antibodies against β 2-glycoprotein I (β 2GPI). The role of β 2GPI (apolipoprotein H, molecular weight about 50 kD) itself in pathophysiology of APS is less clear. Also, little is known about β 2GPI levels in APS patients, and its implications in APS diagnosis.

Aims: To assess possible clinical utility of serum β 2GPI levels in patients with antiphospholipid syndrome

Methods: The study included 40 samples from patients with primary APS ($n = 20$) and secondary APS ($n = 20$) and 20 samples from healthy volunteers (sex- and age- matched). The level of β 2GPI was determined using Human Beta-2-glycoprotein I ELISA Kit (EIAab, China). aCL and anti- β 2GPI antibodies in 2 classes (IgG and IgM) were determined using enzyme-linked immunosorbent assay (QUANTA Lite aCL, α β 2GPI ELISA; INOVA Diagnostics, USA). Lupus anticoagulant (LA) was assessed according to ISTH SSC 2009 guidelines.

Results: We observed that the levels of β 2GPI were significantly higher in the APS group (0.978 ng mL^{-1} ; range $0.043\text{--}12.479$) as compared to the controls (0.349 ng mL^{-1} ; range $0.043\text{--}1.298$), $P = 0.034$. Additionally, in PAPS group the level of β 2GPI was higher (1.486 ng mL^{-1} ; range $0.105\text{--}12.479$) than in the SAPS group (0.471 ng mL^{-1} ; range $0.043\text{--}2.802$), $P = 0.0154$. We also noted that PAPS patients classified as triple-positive (LAC+, aCL IgG/IgM+, and α β 2GPI IgG/IgM+) had higher levels of β 2GPI (2.109 ng mL^{-1} ; range $0.345\text{--}12.479$) compared to double-positive and single-positive patients (0.863 ng mL^{-1} ; range $0.105\text{--}4.434$) but this results were not significant.

Conclusion: Our results show that the serum concentration of β 2GPI in APS patients is higher than healthy controls. The highest β 2GPI levels were observed in triple-positive patients with primary antiphospholipid syndrome

Disclosure of Interest: None declared.

PO070-WED

The association of 3 STAT4 single nucleotide polymorphisms and antiphospholipid syndrome

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Background: Alterations in the signal transducer and activator of transcription (STAT)4 pathway may lead to autoimmune disorders. Some of the single nucleotide polymorphisms (SNP) in the STAT4 gene have been associated with vascular events and the presence of antiphospholipid antibodies in patients with systemic lupus erythematosus (SLE).

Aims: To investigate the association of 3 SNPs in the STAT4 gene: rs7582694 C/G, rs10181658 G/C and rs11684030 G/A with primary and secondary antiphospholipid syndrome (PAPS and SAPS).

Methods: The genotyping was performed in 395 (97 with PAPS, 83 with SAPS, 133 with SLE, and 82 with other autoimmune diseases) patients and 150 healthy controls from southern Poland. The Bioethics Committee approved the study and informed consent was obtained from all the participants in accordance with the Declaration of Helsinki. Identification of STAT4 SNPs was performed using polymerase

chain reaction-restriction fragment length polymorphism. Statistical analysis was done using SAS program.

Results: The genotypes of the SNPs in patients and controls conformed the Hardy-Weinberg equilibrium. The prevalence of STAT4 rs11684030 G/A was similar in all patients and controls. The frequency of C allele of rs7582694 was higher among PAPS and SAPS patients compared to controls (35 vs. 26% and 41.6 vs. 26%, OR=0.65, 95% CI [0.44–0.96], $P = 0.03$ and OR=0.5, 95% CI [0.33–0.74], $P = 0.0008$; respectively). The frequency of G allele of rs10181658 was also higher among PAPS and SAPS compared to controls (35 vs. 26.3% and 40.1 vs. 26.3%, OR=1.5, 95% CI [1.02–2.23], $P = 0.04$ and OR=1.94, 95 CI [1.3–2.9], $P = 0.001$; respectively). The frequencies of C and G alleles of rs7582694 and rs10181658, respectively, were higher in patients with APS compared to other autoimmune diseases ($P < 0.05$), but not SLE ($P > 0.05$).

Conclusion: Higher frequencies of C allele of rs7582694 and G allele of rs10181658 in PAPS and SAPS patients might indicate a genetic predisposition of developing APS.

Disclosure of Interest: None declared.

PO071-WED

Laboratory interpretation of LA test results in the era of target specific oral anticoagulants

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Background: Detection of lupus anticoagulants (LA) is important to diagnose antiphospholipid syndrome (APS). Target specific oral anticoagulants (TSOACs) are used for management of APS-associated thrombosis. The use of TSOACs can influence on LA test, but little information is available on the effects of TSOACs.

Aims: The aim of this study was to describe the effects of TSOACs on LA test results.

Methods: Total 2037 LA tests were done from January 2014 to December 2014. For LA testing, dilute Russell's viper venom time (DRVVT) and silica clotting time (SCT) were evaluated. We retrospectively reviewed diagnosis, drug history and coagulation test results including anti-cardiolipin antibody (ACA) and anti- β 2-GPI antibody of patients with positive LA confirmatory test results.

Results: Nine hundred and ninety-six (48.8%) patients were positive for LA screening test (696 with positive DRVVT test and 786 with positive SCT test). 193 (9.5%) patients were positive for LA confirmatory test. Among them, only DRVVT positive were 125 (64.8%), only SCT positive were 27 (14.0%), and both DRVVT and SCT positive were 41 (21.2%). Diagnoses of positive LA confirmatory test patients were non-malignant disorders ($n = 174$) including APS ($n = 54$) and other autoimmune disorders ($n = 80$), and malignant disorders ($n = 19$). Patients with positive in both LA and ACA and/or anti- β 2-GPI antibody tests were 52 (26.9%). In patients with positive only in LA confirmatory tests, 21 patients received rivaroxaban and 1 patient received dabigatran etexilate. Among them ($n = 22$), 17 (77.2%) showed positive only in DRVVT test and 2 (9.1%) showed positive only in SCT test. In comparison, in patients without TSOACs ($n = 119$), 74 (62.2%) showed positive only in DRVVT test and 27 (22.7%) showed positive only in SCT test.

Conclusion: In patients with TSOACs, the positive rates of DRVVT-based LA confirmatory test were increased. The interpretation of sole positive DRVVT-based LA test results should be carefully done with drug history review, especially rivaroxaban.

Disclosure of Interest: None declared.

Antiplatelets Agents III

PO072-WED

Production and purification of versatile enzymes for potential antiplatelet applications

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Background: Snake venom derived bioactive proteins have been extensively studied with regard to their biological functions on haemostasis. Many of them affect platelet aggregation via activation or inhibition.

Aims: In this study recent advances in our laboratory on bioprocess development for novel antithrombotic enzymes purified from *Agkistrodon contortrix* snake venom are presented.

Methods: A series of HPLC steps, such as affinity, ion exchange and gel filtration chromatography, were employed to purify the enzymes. The purified proteins were biochemically characterised by one and two-dimensional gel electrophoresis, western blotting, enzyme activity assays, platelet aggregation assay, and LC-MS/MS. The proteins were routinely checked for proteolytic activities using chromogenic substrates S-2366 and S-2251.

Results: The three enzymes with antiplatelet properties; an L-amino acid oxidase (LAAO), a fibrinolytic enzyme (FE) and a protein C activator (PCA), were purified to homogeneity and identified via three consecutive HPLC steps and MS/MS analysis, respectively. The LAAO, FE and PCA had a molecular mass of 57.2 kDa, 30.5 kDa and 41.8 kDa, respectively. Sequence alignment analysis confirmed that the enzymes were novel proteins despite having high homology to similar proteins previously reported. Inhibitory effect on agonist-induced rabbit platelet aggregation of the FE and PCA were 90% and 85%, respectively. LAAO inhibited aggregation in a dose-dependent manner which was quenched by the addition of catalase, indicating the characteristic H₂O₂ generation. All enzymes exhibited specificity for the chromogenic substrates.

Conclusion: Here shown is the bioprocess development to purify novel enzymes with antiplatelet activities from *A. contortrix* venom. The purified proteins exhibited profound platelet aggregation inhibitory effect with mechanistic insights investigated. Future work to optimise production scale-up is required for further development of these enzymes as potential agents in the management of thrombosis.

Disclosure of Interest: None declared.

PO073-WED

Antiplatelet effects of glyproline peptides by experimental metabolic syndrome

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Background: The platelet ability to enhanced aggregation in blood is observed by metabolic syndrome, atherosclerosis, diabetes and other disorders. Endothelial dysfunction, relax factors decrease and constrict factors increase are important moment in enhance of platelet aggregation. Direct influence of pharmacology drugs may to decrease of pathology enhanced aggregation.

Aims: The study of proline- and glycine-containing (glyproline) peptides AKT₄₋₇PGP (Semax) or AKT₆₋₉PGP influence on ADP-induced platelet aggregation (ADP-PA) by development of experimental metabolic syndrome (MS) of rats.

Methods: The study was carried out on white rats with experimental MS that was induced by a hyper-cholesterol fat-rich diet (FD) and 10% glucose ad libitum during 6 weeks. After that the rats intranasal administrated the glyprolines (100–500 µg/kg) for 10 days by continuation of FD. Blood for measuring of ADP-PA was collected from ani-

mals 20 h after the last peptides injection and at 7 days after peptides withdrawal. All experiments were complied with the Declaration of Helsinki.

Results: It was demonstrated that studied glyprolines decreased ADP-PA 20 h after the last peptides injection and at 7 days after peptides withdrawal. AKT₆₋₉PGP had maximal antiplatelet effect, because ADP-PA in this case was decreased on 40–45% from control level during all time of experiment. Semax lead to decrease of ADP-PA 20 h after the last peptide injection (in dose 100 µg/kg), and this effect was observed at 7 days after Semax withdrawal too (in dose 100 µg/kg).

Conclusion: Thus our results showed that glyproline peptides have protective antiplatelet effects against enhanced rat platelet aggregation resulting from hyper-cholesterol fat-rich diet. These effects were preserved after peptides withdrawal, that indicate prolonged effects of peptides. We conclude that Semax and AKT₆₋₉PGP may be used as antiplatelet drugs by development of metabolic syndrome.

Disclosure of Interest: None declared.

PO074-WED

Platelet transfusion reverses bleeding evoked by triple anti-platelet therapy including vorapaxar, a novel platelet thrombin receptor antagonist

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Background: Vorapaxar is a novel protease-activated receptor-1 (PAR-1) antagonist recently approved for the reduction of thrombotic cardiovascular events in patients with a history of myocardial infarction or with peripheral arterial disease. Patients who received vorapaxar in addition to standard of care antiplatelet therapy had an increased incidence of major bleeding events compared with placebo.

Aims: To assess whether platelet transfusion can restore hemostasis in primates on triple antiplatelet therapy.

Methods: Template bleeding times were assessed concurrently in the buccal mucosa, finger pad, and distolateral tail of anesthetized cynomolgus macaques to evaluate bleeding with vorapaxar as either monotherapy or in combination with aspirin or aspirin and clopidogrel. Reversal of increased bleeding time was studied by transfusing fresh human platelet rich plasma to triple anti-platelet therapy treated monkeys.

Results: Aspirin (5 mg kg⁻¹, IV) or vorapaxar (1 mg kg⁻¹, PO) alone had no significant effect on bleeding times in the three vascular beds examined. A modest (< 2-fold) increase in bleeding time was achieved in the three beds with the dual combination of aspirin and vorapaxar. Major increases in bleeding time were achieved in the three beds with the triple combination of aspirin (5 mg kg⁻¹, IV), vorapaxar (1 mg kg⁻¹, PO), and clopidogrel (1 mg kg⁻¹, PO). Transfusion of fresh human platelet rich plasma, but not platelet poor plasma, reversed the increase in bleeding time in the triple therapy group.

Conclusion: Transfusion of human platelets may be a viable approach in situations requiring a rapid reversal of platelet function in individuals treated with triple anti-platelet therapy that includes vorapaxar.

Disclosure of Interest: T.-Q. Cai Employee of: Merck & Co., Inc, A. Wickham Employee of: Merck & Co., Inc, G. Sitko Employee of: Merck & Co., Inc, M. Michener Employee of: Merck & Co., Inc, R. Raubertas Employee of: Merck & Co., Inc, L. Handt Employee of: Merck & Co., Inc, M. Chintala Employee of: Merck & Co., Inc, D. Seiffert Employee of: Merck & Co., Inc, M. Forrest Employee of: Merck & Co., Inc

PO075-WED

The antipsychotic clozapine is a potent antiplatelet agent

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Background: Cardiovascular disease (CVD) is a leading cause of mortality in patients with schizophrenia and increased platelet reactivity has been observed in this cohort. Antipsychotics used to treat schizophrenia are believed to increase cardiovascular risk due to their adverse metabolic effects. Paradoxically, epidemiological studies have suggested that antipsychotic therapy with clozapine is associated with reduced cardiovascular mortality.

Aims: The study aimed to compare the effect of typical (haloperidol) and atypical (clozapine) antipsychotics on platelet function.

Methods: Platelet function was assessed using assays of aggregation and activation.

Results: *In vitro*, haloperidol and clozapine inhibited low dose ADP (5 μ M) induced platelet aggregation (500 μ M Clozapine: $24 \pm 5\%$, 500 μ M Haloperidol: $22 \pm 2\%$, control: $84 \pm 2\%$, $P < 0.05$). However, clozapine but not haloperidol inhibited aggregation in response to high dose ADP (10 μ M), low dose convulxin (0.1 μ g mL⁻¹), and pathological shear stress (10,000 s⁻¹). To determine if the antiplatelet effect of clozapine was seen *ex vivo*, platelet function was assessed in schizophrenia patients. Platelet aggregation (20 μ M ADP) was reduced in blood from patients taking clozapine ($35 \pm 9\%$, $n = 4$, $P < 0.05$) compared to controls ($72 \pm 13\%$, $n = 39$). This reduced aggregation response was of similar magnitude to CVD patients taking the P2Y₁₂ antagonist prasugrel ($18 \pm 7\%$, $n = 6$, $P < 0.05$). Platelet function was also measured using a novel assay based on the decrease in adhesion to fibrinogen spots as platelets aggregate. In response to 20 μ M ADP, platelet aggregation was lower in clozapine ($37 \pm 26\%$, $n = 8$) treated patients compared to those on intra-muscular preparations of other anti-psychotics ($79 \pm 7\%$, $n = 7$), reflecting the inhibition of platelet function in clozapine treated patients. **Conclusion:** This study demonstrates that clozapine is a potent inhibitor of platelet function both *in vitro* and *ex vivo*. This antiplatelet effect likely contributes to the reduced incidence of CVD associated with clozapine therapy.

Disclosure of Interest: None declared.

PO076-WED

An *in vitro* transfusion model for the assessment of platelet function recovery following exposure to triple anti-platelet agents

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Background: Dual antiplatelet therapy (DAPT) with aspirin and a P2Y₁₂ antagonist is a standard-of-care to reduce the risk of thrombosis. As DAPT does not directly target thrombin-dependent platelet activation, PAR-1 antagonist addition to DAPT (i.e., TAPT) may improve the efficacy of antiplatelet therapies, though at the expense of increased bleeding risk. This study, using an *in vitro* transfusion model, evaluated if platelet function loss due to TAPT can be remedied by the administration of drug-naïve platelets.

Aims: To assess *in vitro* the potential of platelet transfusions to recover platelet reactivity in patients that are prescribed TAPT using aspirin, clopidogrel, and vorapaxar.

Methods: To mimic TAPT, platelet-rich plasma (PRP) prepared from consented DAPT patients (DPRP) was incubated with vorapaxar (300 nM), resulting in TPRP. To simulate transfusions, TPRP was mixed with increasing proportions of drug-naïve, normal PRP (NPRP). Platelet function recovery was assessed by light transmission aggregometry (LTA), aggregate morphology, and p-selectin expression.

Results: LTA ($n = 14$) showed that 20% NPRP was required in TPRP to restore 10 μ M ADP platelet reactivity to the level originally observed in DPRP, and 40% NPRP was needed for $> 65\%$ ADP response. A 60% NPRP was required for 40% LTA response with either 15 μ M TRAP or 1.6 mM AA. PAR4 mediated LTA (AY-PGKF, 500 μ M) was unaffected. A linear decrease in single platelets with increasing NPRP and aggregate analyses indicated increasing incorporation of mepacrine-labeled NPRP platelets into the NPRP/TPRP aggregates ($n = 4$). Upon TRAP or ADP activation, the percent of platelets positive and surface density of P-selectin increased linearly in TPRP samples with increasing NPRP ($n = 4$).

Conclusion: This *in vitro* model showed that addition of drug-naïve platelets can be a strategy for reversing the PAR-1 antagonist effects of vorapaxar and for recovering platelet function when vorapaxar is administered in combination with DAPT, such as aspirin and clopidogrel.

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PO077-WED

Effects of single compared to dual anti-platelet therapy on hemostatic system activation – a prospective, randomized, double-blind, placebo-controlled trial in healthy volunteers

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Background: Dual anti-platelet therapy (DAPT) with a P2Y₁₂ receptor antagonist (P2Y₁₂-RA) and aspirin is standard in patients with acute coronary syndrome. Although effective, DAPT causes more bleeding than single anti-platelet therapy with a P2Y₁₂-RA (SAPT).

Aims: To test the hypothesis that SAPT and DAPT inhibit hemostatic system activation to the same extent.

Methods: In a prospective, randomized, double-blind, placebo-controlled trial we treated 88 healthy men with clopidogrel (600 mg loading dose (LD), 150 mg od)/ aspirin (100 mg od) or clopidogrel (same dose)/placebo for 7 days and with ticagrelor (180 mg)/aspirin (300 mg) or ticagrelor (180 mg)/placebo once (LD) with 24 h observational time. Blood was obtained from a microvascular injury (bleeding time incision) at baseline (BL) and at several intervals thereafter. β -thromboglobulin (β -TG, mg L⁻¹) and prothrombin fragment 1.2 (f1.2, nM L⁻¹) were measured by ELISA. The AUC was used to discriminate the difference in effects over time. Data are given as median (interquartile range).

Results: Compared to BL, clopidogrel/aspirin and clopidogrel/placebo reduced β -TG from 2.6 (2.2) to 1.5 (1.1) ($P < 0.0001$) and from 2.6 (1.5) to 1.1 (1.5) ($P < 0.0001$). Ticagrelor/aspirin and ticagrelor/placebo decreased β -TG from 1.7 (1.7) to 0.7 (0.7) ($P < 0.0001$) and from 1.8 (1.3) to 0.8 (0.8) ($P = 0.001$). Ticagrelor inhibited f1.2 generation (+ aspirin from 10 (13) to 6 (7), $P = 0.0002$; + placebo from 10 (10) to 5 (6), $P = 0.003$), but clopidogrel did not. No difference was found

between treatments. As regards AUC, a difference in β -TG or f1.2 was seen neither between clopidogrel/aspirin and clopidogrel/placebo (β -TG: 1390 (121) vs. 1378 (133), $P = 0.7$; f1.2: 1423 (98) vs. 1451 (78), $P = 0.4$) nor between ticagrelor/aspirin and ticagrelor/placebo (β -TG: 146 (19) vs. 153 (20), $P = 0.4$; f1.2: 192 (15) vs. 198 (19), $P = 0.3$).

Conclusion: P2Y₁₂-RA mono-therapy and conventional DAPT inhibit platelet and coagulation activation in the microcirculation to the same extent.

Disclosure of Interest: None declared.

PO078-WED

Development of a receptor occupancy assay for anfibatide, a novel GPIIb antagonist

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Background: Treatment for thrombosis, particularly GPIIb/IIIa antagonists, can cause serious bleeding complications. Unlike GPIIb/IIIa, GPIb initiates thrombus formation at high shear stress, prominent in atherosclerotic vessels. This target could prevent pathological thrombosis, with minimal effect on low shear vessels. Anfibatide is a novel snake venom-derived GPIb antagonist, entering a phase IIa multi-center clinical trial in China in 2015. Appropriate assays are necessary for the pharmacokinetic and pharmacodynamic clinical studies.

Aims: Develop and validate a quantitative flow cytometry-based receptor occupancy assay for anfibatide using healthy donor platelets *ex vivo* and compare this to functional assays.

Methods: Platelets were stained with anti-GPIb monoclonal antibodies or anti-anfibatide antibody. Binding was quantified using the BioCytex Platelet Calibrator kit. The effect of anfibatide on ristocetin-induced platelet aggregation and whole blood aggregation was measured using a Multiplate analyzer.

Results: 69,181 $\pm$ 6242 anfibatide binding sites were detected per platelet at the optimal dose of 16 $\mu\text{g mL}^{-1}$, $n = 3$, with a K_d of $2.5 \pm 0.6115 \mu\text{g mL}^{-1}$. Anfibatide partially inhibited anti-GPIb antibodies VM16d ($52 \pm 2\%$ inhibition, $P = 0.036$) and SZ2 ($23 \pm 1\%$, $P = 0.012$) ($n = 3$). Anfibatide inhibited ristocetin-induced whole blood aggregation in a dose-dependent manner ($P = 0.043$, $n = 3$), correlating with GPIb receptor occupancy (IC_{50} 2.39 $\mu\text{g mL}^{-1}$, CI 1.08–5.31). 10 $\mu\text{g mL}^{-1}$ anfibatide inhibited platelet aggregation in the Multiplate assay by 77.5%, and did not cause platelet activation or loss of single platelets.

Conclusion: A flow cytometry-based receptor occupancy assay was optimised for measuring GPIb receptor occupancy and is suitable for clinical trials. Receptor occupancy by anfibatide correlates with inhibition of ristocetin-induced aggregation in whole blood by anfibatide indicating that there are no spare GPIb receptors.

Disclosure of Interest: None declared.

PO079-WED

Pegylated disintegrin exhibits more potent antithrombotic activity *in vivo*

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Background: The utility of protein drugs to treat diseases is limited by protein degradation *in vivo* and rapid clearance. PEGylation provides one means by which improves protein stability by sterically blocking

protease access, and the increased size of PEGylated proteins reduces renal elimination.

Aims: We used the PEGylated Rhodostomin (PRn) in order to prolong its half-life, and to reduce the antigenicity of Rn in animal models.

Methods: PRn was provided by Dr. Chuang. Human washed platelet and mouse platelet-rich plasma (PRP) were prepared for aggregation assay. The *in vivo* effects of disintegrins on occlusion time were revealed in ferric chloride-induced arterial thrombosis animal model.

Results: In human platelet aggregation assay, native Rn and PRn possess similar inhibition on platelet aggregation induced by collagen and thrombin. In *ex vivo* mouse platelet aggregation assay, mice were injected with the same dosage of Rn or PRn and blood drawn cardiac-punctually. After preparing PRP from mice, the effect of these two disintegrins on collagen-induced platelet aggregation were also compared (55.50 ± 9.69 and 54.66 ± 7.06 nM, respectively). PRn prolonged the *in vivo* half-life from 0.5 h to longer than 2 h. In ferric chloride-induced arterial thrombosis, Rn only significantly prolonged occlusion time at high dosage (0.2 mg kg⁻¹). However, PRn obviously prolonged the occlusion time even given at a lower dosage (0.06 mg kg⁻¹).

Conclusion: PEGlated disintegrin exhibits the advantages of longer t1/2 and better antithrombotic efficacy compared with native disintegrin. Therefore, PEGylated disintegrin derivatives would be the desirable strategy in modifying protein-drugs in future.

Disclosure of Interest: None declared.

PO080-WED

Resistance to aspirin in myeloproliferative neoplasms: is it a reality?

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Background: Myeloproliferative neoplasms (MPN) are associated with an increased risk of arterial thrombosis. Low dose aspirin is recommended in primary and secondary prophylaxis of thrombosis in polycythemia vera (PV) and in secondary prevention in essential thrombocythemia (ET) without specific optimal dose.

Aims: To determine the optimal dose of aspirin to achieve biological efficacy in Philadelphia-negative MPN patients.

Methods: Patients with MPN (without any cytostatic drug or with either pegylated interferon alpha or hydroxyurea) who were treated with low-dose aspirin were enrolled. Resistance to aspirin was determined by platelet aggregation induced by arachidonic acid (1.33 mM) on platelet rich plasma at through level, 24 h after the last intake, and was defined by a maximal platelet aggregation over 20%.

Results: Seventy-seven patients were included (43 PV, 30 ET, 4 myelofibrosis). 53 were treated with aspirin 75 mg day⁻¹ and 24 with aspirin 100 mg day⁻¹. Out of the 53 patients treated with aspirin 75 mg day⁻¹, 12 patients (23%) were resistant to aspirin. An increased dose of 100 mg overcame this biological resistance in 8/10 patients. 2/24 patients under 100 mg day⁻¹ were resistant. 75 mg² per day was required to overcome the biological resistance. No correlation between age, presentation, treatment, thrombotic or bleeding events before inclusion could be observed between the different groups of patients.

Conclusion: At baseline, 23% of the patients were resistant to aspirin 75 mg day⁻¹ vs. 8% with 100 mg day⁻¹. When the aspirin dose was adapted to biological efficacy, only 2.6% of the patients remain resistant to aspirin 100 mg day⁻¹. No bleeding event was observed during the 6-month follow-up. The dose of aspirin of 100 mg day⁻¹ seems the best compromise. A prospective study is required on a larger number

of patients with an extended follow-up period to determine if biological resistance to aspirin is correlated to the occurrence of thrombotic events.

Disclosure of Interest: None declared.

PO081-WED

Pharmacokinetics and pharmacodynamics of APAC, a dual novel anticoagulant and antiplatelet heparin complex

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Background: APAC2 heparin-protein complex is inspired by mast cell-derived heparin proteoglycans which attenuate platelet-collagen interactions under blood flow and reduce platelet thrombosis. In addition to inhibiting platelets, APAC2 is an anticoagulant and arrests thrombus growth in two arterial baboon models (Lassila and Jouppila, STH 2014). Upon local administration APAC2 targets and remains at vascular injury site. *In vitro*, APAC2 inhibits collagen-induced platelet aggregation and procoagulant activity in calibrated automated thrombogram analysis.

Aims: To study the pharmacokinetics (PK) and pharmacodynamics of APAC2 in rats.

Methods: Male Sprague-Dawley rats were dosed with APAC2 or unfractionated heparin (UFH) at 0.13 or 7.3 mg kg⁻¹. Blood samples were drawn pre and post dose at 60 min, 90 min, 6 h, 24 h and 48 h for APTT and PT analysis. Distribution and retention were studied in two rats by PET/CT scan with ⁶⁴Cu-labeled APAC2 or UFH. At 48 h, rats were euthanized and the kidneys, liver, lungs and spleen harvested for ⁶⁴Cu radioactivity counting.

Results: At both 0.13 and 7.3 mg kg⁻¹ (15-fold above target dose), APAC2 and UFH were mainly distributed to the kidneys and liver. APAC2 clearance (T_{1/2}) was rapid, 15 min at 0.13 mg kg⁻¹ and 1 h at 7.3 mg kg⁻¹ while UFH T_{1/2} was 23–28 min at both doses. At 0.13 mg kg⁻¹, APAC2 or UFH had no effect on APTT, but both prolonged APTT upto 6- and 4-fold respectively at 7.3 mg kg⁻¹. APAC2 and UFH resulted in 1.5-fold prolongation of PT only at 7.3 mg kg⁻¹. At the highest dose APTT remained elevated at 90 min while PT reverted. APTT and PT were back to normal after 6 h.

Conclusion: APAC2 and UFH mainly accumulated in the kidneys and liver following the heparin elimination route. APAC2 had 2.5-fold clearance time over UFH suggesting some retention. APAC2 prolonged APTT, while PT was only modestly elevated suggesting minimal impact on extrinsic pathway. The PK profile of APAC2 appears beneficial.

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PO082-WED

Inhibition of thrombin generation by apixaban or rivaroxaban, in a cell-based model of coagulation primed by platelets

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Background: Platelets contribute to the pathogenesis of thrombotic and ischemic events in arterial territories. Exposure of tissue factor at

sites of disrupted atherosclerotic plaques promotes local thrombin generation, enhancing further recruitment of platelets into occlusive thrombi. Direct oral anticoagulants (DOACs) inhibit thrombin generation; however, their effect on platelet-mediated procoagulant activity has not been explored.

Aims: Design a cell-based model of coagulation to assess the contribution of platelets to thrombin generation. Evaluate the effects of apixaban (APIX) and rivaroxaban (RIV) in this system.

Methods: Platelets were used as the source of anionic phospholipids to enhance thrombin generation in a modified fluorometric assay. A 10% of plasma ensured the presence of coagulation factors to support thrombin generation. Samples were incubated with APIX or RIV at concentrations 4–10 times lower than their C_{max} for prevention of thrombotic complications in atrial fibrillation. Thrombin generation triggered by tissue factor, FVIIa and calcium, was recorded for 90 min.

Results: We have characterized a model with an enhanced sensitivity to detect the contribution of platelets to thrombin generation. Low concentrations of APIX and RIV inhibited the potential of the system to generate thrombin. Times to start and reach the maximum thrombin peak were delayed by both DOACs in a dose-response form ($P < 0.05$). The maximum amount of thrombin generated was also decreased ($P < 0.05$).

Conclusion: Our cell-based model of coagulation primed by platelets facilitates the evaluation of the contribution of platelets to thrombin generation. Concentrations of APIX and RIV lower than their usual therapeutic C_{max} significantly affect the contribution of platelets to thrombin generation. This model could be useful to assess optimal concentrations of the DOACs that could be effective in the prevention of arterial thrombotic risk.

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PO083-WED

How should we measure the effect of persantine using thromboelastography? Correlation and agreement between percent ADP inhibition AND ADP Net G

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Background: Controversy exists as to whether percent ADP (%ADP) inhibition or ADP Net G should be used as the primary laboratory target to adjust the dose of platelet inhibitors in children with ventricular assist devices (VAD).

Aims: To examine the agreement between these two values derived from Thromboelastography with Platelet Mapping® (TEG/PM) with respect to dose-adjustment recommendations.

Methods: All pediatric patients with a VAD between 2006 and 2014 with TEG/PM data available at two pediatric VAD centers were identified using their respective hospital databases and merged. For each specimen, the %ADP inhibition result (target 70–95%) was compared to the 'ADP NET G' value (target 4–8), defined as the product of the baseline G (citrate kaolin with heparinase) and (1

Results: A total 665 TEG/PM specimens in 51 children, median age 11 months (IQR 6 mo, 5 years), median weight 8.9 kg (IQR 6.2, 17.7); 29% had congenital heart disease, 8% with single-ventricle, 49% received a temporary circulatory support device pre-implant, and 65% had an LVAD. Mean %ADP inhibition was $49\% \pm 35\%$ (SD) and mean ADP Net G 5 ± 3.9 with a Pearson's correlation of 0.78 for simultaneous values. Using %ADP inhibition of 70–95 as the target, 15.3% were classified as in-range, 66% sub-therapeutic and 18% supra-therapeutic; using ADP Net G of 4–8 as the target, 43.5% of values were classified as in-range, 41% sub-therapeutic and 14% supra-therapeutic. Correlation between the two classification systems was good (R 0.71, $P < 0.001$); agreement on dosing recommendation was poor (Kappa 0.39, $P < 0.001$) related to the NET G value classifying significantly more patients as in-range.

Conclusion: %ADP inhibition and ADP Net G values correlate well however there is poor agreement on dosing recommendations when an ADP Net G target of 4–8 is compared to a %ADP inhibition of 70–95%. Further research is needed to determine the optimal ADP target for pediatric VAD patients.

Disclosure of Interest: P. Massicotte Consultant for: NIH/NHLBI, J. Conway: None declared, L. May: None declared, H. Buchholz: None declared, C. Lo: None declared, A. Bruce: None declared, T. Tesoro: None declared, D. Rosenthal: None declared, C. Almond: None declared.

PO084-WED

NSAIDs *in vivo* treatment and MRP4 expression in human platelets

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Background: Platelet MRP4 overexpression has a role in reducing Aspirin action in patients after by-pass surgery. Aspirin induces platelet MRP4 over-expression, through megakaryocytes genomic modulation, consequent to the activation of the nuclear receptor PPAR- α .

Aims: Aim of our work is to verify whether other Non-Steroidal Anti Inflammatory Drugs (NSAIDs) are able to enhance platelet MRP4 expression and evaluate platelet function in patients who overexpress MRP4.

Methods: In human megakaryoblastic cell line (DAMI cells), treated with selective COX-2 inhibitors (Celecoxib 25 μ M) and traditional NSAIDs (Diclofenac 50 μ M and Naproxen 50 μ M) for 48 h, we evaluated MRP4-mRNA (RT-Q-PCR). Osteoarthritis patients ($n = 17$), who reported to take NSAIDs in two occasions per week for at least four continuous weeks, and a control population ($n = 26$), who didn't take any NSAIDs in the previous month, were enrolled. In these patients, we evaluated platelet MRP4 amount, by both mRNA level (RT-Q-PCR) and protein expression (Western Blot). Platelet aggregation (Born's method) in response to ADP (0.8–2 μ M) was also performed.

Results: DAMI cells treated with Celecoxib, Diclofenac and Naproxen after 48 h showed a significant increase in MRP4 mRNA expression compared to the mock culture (fold increase 1.43–1.5–1.62 respectively). In Osteoarthritis patients we found a significant increase in both MRP4 mRNA expression (fold increase 3.2) and in MRP4 protein expression (fold increase 1.4) and a significant increase in ADP-induced platelet aggregation (0.8 μ M) ($P < 0.05$) compared to the control population.

Conclusion: NSAIDs treatment induces MRP4 overexpression after *in vivo* and *in vitro* treatment. Osteoarthritis patients, who overexpress MRP4, show platelet hyper-reactivity.

Disclosure of Interest: None declared.

PO085-WED

The effectiveness of P2Y₁₂ receptor blockade is strongly determined by the presence of endothelial Mediators prostacyclin and nitric oxide

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Background: P2Y₁₂ receptor antagonists (e.g. prasugrel) and aspirin are prescribed as dual anti-platelet therapy (DAPT) to patients at risk of thrombosis. P2Y₁₂ receptor antagonists block the effects of pro-aggregatory ADP. Blockade of platelet P2Y₁₂ receptors may also increase the synergistic inhibition of platelets driven by the endothelial mediators nitric oxide (NO) and prostacyclin (PGI₂).

Aims: To test the hypothesis that inter-patient variances in endothelial function and the productions of NO and PGI₂ could be crucial determinants of the efficacy of DAPT.

Methods: Healthy male volunteers ($n = 8$ each) received either prasugrel (10 mg), aspirin (75 mg) or DAPT for 7 days. Platelet responses to TRAP-6 (25 μ M) in the presence of PGI₂ (1 nM), NO (100 nM), PGI₂+NO or vehicle were assessed by light transmission aggregometry before and during therapy. *In vitro*, experiments were performed ($n = 4$) with blood incubated with aspirin (30 μ M) plus prasugrel active metabolite (PAM; 6, 3 or 1.5 μ M) to represent maximal and partial P2Y₁₂ blockade.

Results: *Ex-vivo*, PGI₂+NO had no effect on platelet aggregation prior to therapy. However, PGI₂+NO inhibited aggregation during aspirin therapy (control, $58 \pm 8\%$; PGI₂+NO, $28 \pm 9\%$; $P < 0.05$), and particularly inhibited aggregation during prasugrel therapy ($63 \pm 3\%$ vs. $7 \pm 3\%$; $P < 0.05$) and DAPT ($67 \pm 5\%$ vs. $19 \pm 6\%$; $P < 0.05$). *In vitro*, maximum P2Y₁₂ (PAM, 6 μ M) inhibition significantly ($P < 0.05$) reduced aggregation in all conditions. However, partial P2Y₁₂ (PAM, 1.5 μ M) inhibition only reduced aggregation in the presence of PGI₂+NO ($67\% \pm 5$ vs. $35 \pm 15\%$; $P < 0.05$).

Conclusion: PGI₂ and NO synergize with P2Y₁₂ blockade to produce powerful platelet inhibition. This strongly suggests that *in vivo* platelet reactivity during DAPT is a function of the level of P2Y₁₂ receptor blockade and the levels of inhibitory endothelial mediators. We suggest that assessment of endothelial function alongside *ex vivo* platelet function would provide improved prediction of thrombotic outcomes.

Disclosure of Interest: None declared.

PO086-WED

Comparison of the biocytex and the vaspfix assays of VASP phosphorylation in coronary-artery disease patients treated with clopidogrel plus aspirin

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Background: There is high inter-individual variability in response to clopidogrel, a drug that antagonizes the platelet P2Y₁₂ receptor (P2Y₁₂R) for adenosine diphosphate (ADP). The pharmacological effect of clopidogrel can be tested by measuring the phosphorylation of vasodilator stimulated phosphoprotein (VASP-P) with Biocytex (Diagnostic Stago, FR), which reflects the ability of ADP/P2Y₁₂R to inhibit adenylyl cyclase. It utilizes prostaglandin (PG) E₁ to stimulate adenylyl cyclase and requires platelet fixation and permeabilization steps. A novel bead assay for VASP-P, VASPFix (Platelet Solutions, UK), uses Iloprost (a PGI₂ mimetic) instead of PGE-1 and a lysis step instead of permeabilization, to facilitate antibody binding.

Aims: To compare VASPFix with Biocytex assay and with two tests of platelet aggregation (PA) in CAD patients receiving dual antiplatelet therapy (DAPT) of aspirin (100 mg day⁻¹) plus clopidogrel

Methods: We enrolled 24 patients with stable CAD on chronic DAPT; 15 stable CAD patients on aspirin monotherapy and 15 healthy subjects as controls. PA induced by ADP (5 µM) was measured by light transmission aggregometry (LTA) and impedance aggregometry (Multiplate).

Results: VASPFix platelet reactivity index (PRI) in CAD patients on DAPT (50 ± 22%) was significantly lower compared to aspirin monotherapy (85 ± 7.9%) or healthy subjects (86 ± 8.6%). VASPFix and Biocytex showed no significant difference in mean PRI and moderate-good correlation (Pearson's $r = 0.625$, $P < 0.001$) in patients on DAPT and both correlated moderately with PA measured by LTA ($r = 0.548$ and $r = 0.464$, $P < 0.001$) and poorly with PA measured by Multiplate ($r = 0.306$ and $r = 0.316$, $P < 0.05$). Agreement between VASPFix and Biocytex was highly dependent on cut-off values used to define responders to clopidogrel (Cohen K ranged between 0.196 and 0.882 PRI cut-off between 30 and 60%).

Conclusion: Despite the theoretical advantages of VASPFix, we obtained comparable results using VASPFix and Biocytex.

Disclosure of Interest: None declared.

Arterial Vascular disorders – clinical III

PO087-WED

Local parameters of hemostasis activation in the fibrillating atrium

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Background: Atrial fibrillation (AF) is a common cardiac arrhythmia associated with a high risk of stroke.

Aims: To identify hemostasis abnormalities, which are associated with AF and increase the risk of thromboembolism.

Methods: Patient group consisted of 22 patients with AF, control group included 10 individuals with other types of supraventricular tachycardias undergoing transcatheter radiofrequency ablation. Blood samples of both groups were drawn from the femoral vein, left atrium and left atrial appendage after the insertion of the catheter. Fibrinogen, factor VIII (FVIII) activity, von Willebrand factor (VWF) antigen levels, sVCAM-1, factor XIII (FXIII) activity, thrombin-antithrombin (TAT) complex, quantitative fibrin monomer (FM), activated factor VII-antithrombin (FVIIa-AT) complex, thrombin generation (TG) assay were carried out from all blood samples. The study was approved by the Regional Ethics Committee and informed consent was obtained.

Results: Levels of FVIII and VWF were elevated in the samples obtained from the femoral vein and left atrium of AF patients as compared to controls. Heightened values were not associated with CRP elevation. FVIII and VWF levels showed good correlation in all sample types, suggesting that they are in complexed form. sVCAM-1 levels were also elevated in the femoral vein and left atrium samples of AF patients. TAT complex and FM levels were elevated in the femoral vein samples of all AF patient and control samples. Both parameters were significantly elevated in the left atrium and the left appendage as compared to femoral vein samples in case of both groups. No significant difference was found between sample types and patient groups in case of FXIII, FVIIa-AT, fibrinogen levels and the TG test.

Conclusion: AF patients have elevated FVIII and VWF levels which might be attributed to endothelial damage. Local hemostasis activa-

tion in the left atrium and appendage could be demonstrated by elevated TAT complex and FM levels.

Disclosure of Interest: None declared. Funding: OTKA K109712, PD111929.

PO088-WED

The assesment of endothelial progenitor cells in patients with st elevation myocardial infarction under fibrinolytic therapy or primary percutaneous coronary intervention

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Background: Endothelial progenitor cells (EPCs) have a role in repairing the vascular system during acute myocardial infarction (AMI). EPCs are mobilized from bone marrow to peripheral blood to join for recovery of the injured heart muscle early after AMI.

Aims: This study was planned to investigate the effects of two distinct approaches (thrombolytic therapy and primary percutaneous coronary intervention [PCI]) on the release of EPCs in peripheral circulation in patients with AMI.

Methods: We enrolled 25 eligible patients (10 fibrinolytic treatment and 15 PCI), and 10 healthy volunteers as the control group. Demographic characteristics and laboratory values for all patients and healthy volunteers were recorded. EPC counting was done using flow cytometry (CD34⁺, KDR⁺ and CD133⁺ cells) (for patients also in first and fifth days after treatment).

Results: There was no difference in pretreatment EPC counts. However, first and fifth day EPC counts were significantly higher. Also, the fifth day EPC counts were significantly higher than the first day counts. The EPC counts were similar in treatment groups (Table). There was a positive correlation between the EPC count and cardiac marker (creatinine kinase MB, troponin T/I) and inflammatory marker (CRP and leucocyte count), despite a negative correlation between EPC count and left ventricular ejection fraction.

EPC	Control (n:10)	Fibrinolytic (n:10)	PCI (n:15)
Pretreatment	20.10 ± 5.42	20.50 ± 16.35	26.80 ± 9.54
Day 1		35.00 ± 14.25 ^a	37.53 ± 12.85 ^b
Day 5		44.10 ± 19.74 ^{c,d}	49.86 ± 16.43 ^{e,f}

a-b-c: $P < 0.01$ compared to pretreatment, d: $P < 0.0001$ compared to first day, e: $P < 0.0001$ compared to pretreatment, f: $P < 0.05$ compared to first day.

Conclusion: We concluded that EPC count increased evidently in AMI patients within days. Cardiac markers, inflammatory markers and left ventricular ejection fraction were found to be related with EPC counts. Two types of revascularization therapy presented similar EPC counts, suggesting that long-term studies are necessary.

Disclosure of Interest: None declared.

PO089-WED

rhADAMTS13 treatment ameliorates cardiac long-term remodeling after ischemia-reperfusion injury

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Background: Myocardial ischemia/reperfusion injury (MIRI) occurs when acutely obstructed coronary vessels are reopened by percutaneous coronary intervention. It significantly contributes to the overall

tissue damage after myocardial infarction. Despite many promising approaches, no specific effective treatment has been established to ameliorate the reperfusion injury in patients with acute MI. Only recently our lab has shown that MIRI in mice is ameliorated after 24 h by infusion of rhADAMTS13, which cleaves ultra-large VWF and, hence, decreases inflammatory cell recruitment and microvessel-obstruction. The role of rhADAMTS13 treatment in cardiac long-term outcome, however, has not yet been addressed in this context.

Aims: To study the long-term effects of a single rhADAMTS13 injection after MI by functional, clinical and histological analysis in a mouse model of MIRI.

Methods: Wild-type C57Bl/6J mice underwent a surgical mouse model of MIRI (1 h occlusion of the left anterior descending artery followed by reperfusion) or sham. RhADAMT13 or vehicle infusion was performed before reperfusion via retro-orbital intravenous administration. Echocardiographic examination of heart function and dimensions was performed after 2 and 4 weeks. After 4 weeks plasma was collected for BNP-ELISA and hearts were harvested for histological analysis of infarct areas.

Results: Significant preservation of cardiac function in rhADAMTS13 treated mice compared to vehicle treated mice could be observed after 4 weeks ($P = 0.0002$). Left ventricular dilation was partly reduced and scar tissue was decreased by about 30% ($P = 0.013$) in the rhADAMTS13 treated group.

Conclusion: Here we show that a single injection of rhADAMTS13 improves long-term outcome after MIRI in mice, likely due to reduction of cardiac tissue death and scarring. Our study further supports the therapeutic potential of rhADAMTS13 for MI patients in the future.

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PO090-WED

Risk factors for coronary artery disease in young Iranian: results from the Milano-Iran (MIRAN) study

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Background: Risk factors for coronary heart disease (CHD) are not well studied in Iranian young adults.

Aims: To investigate cardiovascular risk factors for CHD in young Iranian males and females.

Methods: MIRAN is a cohort study of young subjects (males < 45 years, females < 55 years) who underwent diagnostic coronary angiography (CA). We performed a nested case-control study, contrasting subjects with hemodynamically relevant lesions at CA and no past history of CHD with subjects with no lesions. Odds ratios (OR), as measure of association, were calculated by logistic regression for several cardiovascular risk factors and adjusted for age. To assess to what extent the burden of CHD can be attributed to a risk factor, we calculated the population attributable fractions (PAF) by the formula [(proportion of exposed cases)*((OR-1)/OR)]. All analyses were stratified by sex.

Results: We enrolled 1019 cases (35% males) and 1441 controls (26% males). Mean age was 46.6 for cases and 45.6 for controls. In males,

relevant risk factors for CHD were smoking (OR 2.1, 95% Confidence Intervals 1.5–2.9), opium addiction (OR 2.1, 95% CI 1.4–3.2), hyperlipidaemia (OR 1.9, 95% CI 1.4–2.6), hypertension (OR 1.7, 95% CI 1.2–2.4), diabetes (OR 1.6 1.0–2.8) and body mass index > 30 (OR 1.6, 95% CI 1.1–2.5). Conversely, in female diabetes was strongly associated with CHD (OR 4.1, 95% CI 3.3–5.1), followed by hypertension (OR 2.6, 95% CI 2.1–3.1), hyperlipidaemia (OR 2.2, 95% CI 1.7–2.7) and smoking (OR 1.4, 95% CI 0.9–2.0). Of those risk factors, the ones with the greatest PAF were, in males, smoking (PAF 0.33, 95% CI 0.21–0.41) and hyperlipidaemia (PAF 0.33, 95% CI 0.20–0.43), and in females, hypertension (PAF 0.42, 95% CI 0.36–0.46), diabetes (PAF 0.38, 95% CI 0.35–0.40) and hyperlipidaemia (PAF 0.42, 95% CI 0.32–0.48).

Conclusion: Risk factors for CHD differ between young Iranian males and females. These results could be useful in planning CHD prevention strategies.

Disclosure of Interest: None declared.

PO091-WED

Blocking heteromerization of platelet chemokines CCL5 and CXCL4 reduces inflammation and preserves heart function after myocardial infarction

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Background: Myocardial infarction (MI) is among the most common causes of death in developed countries and its incidence is still increasing. Finding new strategies to prevent and treat this threatening clinical event is thus of high priority. Inhibition of CCL5 was shown to have beneficial effects on the outcome of experimental MI in mice, yet might be accompanied by adverse immunologic side effects. In a previous study, we have demonstrated a pathophysiologic relevance for the heteromer formation of CCL5 and CXCL4 in the progression of atherosclerosis.

Aims: To evaluate a specifically designed compound (MKEY) that blocks the CCL5-CXCR4 interaction in a mouse model of myocardial ischemia/reperfusion (I/R).

Methods: To examine the effect of MKEY in healing following I/R, 8 week-old male mice were intravenously treated with MKEY or scrambled control (sMKEY) from 1 day before, until up to 7 days after I/R. Myocardial function was evaluated using echocardiography and intraventricular pressure measurements and tissue viability, scar formation and leukocyte infiltration was assessed by histology.

Results: MKEY treatment resulted in a significant decrease in infarction size and preserved heart function as compared to sMKEY-treated animals. Moreover, MKEY treatment significantly reduced the inflammatory reaction following I/R, as revealed by specific staining for neutrophils and monocyte/macrophages.

Conclusion: Disrupting chemokine heterodimers during myocardial I/R might have clinical benefits, preserving the therapeutic benefit of blocking specific chemokines, and in addition, reducing the inflammatory side effects while maintaining normal immune defense.

Disclosure of Interest: None declared.

PO092-WED

Increased clot formation in the absence of increased thrombin generation in patients with peripheral arterial disease

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Background: In peripheral arterial disease (PAD), arterial atherosclerotic lesions and coagulation factors interact, causing activation of the hemostatic system which contributes to atherosclerotic progression and atherothrombotic events.

Aims: To assess the overall coagulation status in patients with PAD.

Methods: Blood was collected from 40 PAD-patients ($n = 20$ with a cardiovascular event within 1 year after diagnosis and $n = 20$ without event) and 40 healthy controls. ROTEM experiments were triggered with 35 pM tissue factor (TF) with/without 175 ng mL⁻¹ tissue Plasminogen Activator (tPA). Thrombin generation (TG) by means of the Calibrated Automated Thrombography was determined in platelet poor plasma (PPP) using a 1 or 5 pM TF trigger. TG in a thin layer of whole blood (WB) was measured using a 0, 0.5 or 2.5 pM TF trigger.

Results: The levels of leucocytes, von Willebrand Factor (vWF) antigen, fibrinogen and D-dimer were significantly increased in PAD-patients. In the ROTEM-assay, PAD-patients showed faster clot formation and increased clot firmness. Fibrinolysis parameters measured with tPA-ROTEM were comparable between PAD-patients and controls. TG-parameters in PPP and WB showed no differences between patients and healthy controls. The endogenous thrombin potential (ETP) in WB was decreased in PAD-patients with a cardiovascular event compared to PAD-patients without such an event when TG was triggered with 0 pM and 2.5 pM TF (800 vs. 935 nM min, $P = 0.013$).

Conclusion: The increase in levels of vWF, fibrinogen, D-dimer and inflammation markers, and the increased ability to form a stable clot indicate a hypercoagulable state in PAD-patients. However, the TG capacity was not increased in these patients. This could be due to consumption of the coagulation factors, since we observed a decrease in ETP in PAD-patients with a cardiovascular event compared to PAD-patients without such an event. Overall, our results confirm the importance of the inflammation-coagulation axis in PAD.

Disclosure of Interest: None declared.

PO094-WED

Major adverse cardiovascular events in patients with acute coronary syndrome undergoing coronary angioplasty: role of NADPH oxidase P22PHOX gene polymorphisms

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Background: Reactive oxygen species (ROS) play a primary role in atherosclerosis. The major source of ROS in artery wall is related to NADPH oxidase activity, where they promote oxidative stress and plaque progression/instability. A higher expression of p22phox protein, a subunit of NADPH oxidase coded by *CYBA* gene, was observed in human atherosclerotic coronary arteries.

Aims: We aimed to evaluate the role of rs4673, rs7195830, rs9932581 *CYBA* polymorphisms on the occurrence of major adverse cardiovascular events (MACE) at 2-year follow-up.

Methods: In a cohort of $n = 1361$ patients with acute coronary syndrome (ACS) undergoing coronary angioplasty we genotyped the 3 polymorphisms by Taqman real time PCR technology.

Results: The status of A allele carriers (AA+AG) of the rs9932581 polymorphism in the *CYBA* promoter was associated with MACE protection (59.3% MACE vs. 69.1% noMACE, $P = 0.03$). At the multivariate logistic regression analysis, adjusted for known determinants of MACE, the status of rs9932581 A allele carrier resulted a significant and independent protective factor for MACE (OR=0.634, 95% CI 0.422–0.95, $P = 0.028$). The genotype distribution of rs4673 polymorphism did not show a statistically significant difference between patients with and without MACE, even if the prevalence of T allele carriers (CT+TT) was lower MACE patients. No significant association with MACE occurrence was observed for rs7195830 polymorphism.

Conclusion: Our data suggested that polymorphism rs9932581 in *CYBA* gene plays a crucial role in susceptibility to MACE in high risk vascular patients.

Disclosure of Interest: None declared.

PO095-WED

Thrombogenicity according to the vascular site of atherosclerosis disease

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Background: Atherosclerosis is the underlying mechanism shared between patients with abdominal aortic aneurysm (AAA), peripheral arterial obstructive disease (PAOD) and carotid obstruction (CO). They are all at increased cardiovascular risk due to acute atherothrombotic events.

Aims: The aim of our study is to compare thrombogenicity between groups.

Methods: We investigated 162 patients under preoperative evaluation for vascular surgery (61 AAA, 57 PAOD, and 44 CO). All patients received aspirin therapy. Thrombin generation was assessed as endogenous thrombin potential (ETP), with and without thrombomodulin, and the ETP ratio (with-to-without thrombomodulin) expressed a procoagulant-imbalance. We tested platelet aggregation in response to arachidonic acid (Plat Aggr AA) on an impedance aggregometer, for aspirin responsiveness evaluation. The local ethics committee approved the study and patients provided informed consent.

Results: ETP and ETP ratio were lower in AAA in comparison to PAOD and CO: ETP 433.75 ± 436.35 , 707.74 ± 471.05 , $679.68 \text{ nM} \cdot \text{min} \pm 386.35$; $P = 0.002$; ETP ratio 0.29 ± 0.25 , 0.45 ± 0.27 ; 0.48 ± 0.29 ; $P = 0.001$, respectively. Plat Aggr AA was similarly lower in AAA and CO than in PAOD: 5.13 ± 5.10 ; 4.32 ± 5.30 ; $8.09 \Omega \pm 6.68$, $P = 0.002$. Age (69 ± 9 , 65 ± 10.69 years ± 10 ; $P = 0.029$), male sex (98.5%, 56.1%, 65.9%; $P < 0.001$), diabetes (14.7%, 42.1%, 31.8%; $P = 0.004$), platelet count (203.984 ± 59.661 , 281.702 ± 97.079 , 226.909 ± 78.303 ; $P < 0.001$) and creatinine (1.55 ± 1.27 , 1.08 ± 0.45 , $1.34 \text{ mg dL}^{-1} \pm 0.63$; $P = 0.018$) were different between AAA, PAOD and CO. After adjustment for sex, diabetes, creatinine and platelet count, ETP ($P = 0.003$) and ETP-ratio ($P < 0.000$) remained significantly lower in AAA. In the other hand, aspirin responsiveness was significantly lower in PAOD ($P = 0.021$), adjusted for age, sex and diabetes.

Conclusion: Abdominal aortic aneurysm patients have a less thrombogenic status than other vascular patients. Differences in coagulation

profile between patients with AAA, PAOD and CO may be a useful tool for therapeutic guidance.

Disclosure of Interest: None declared.

PO096-WED

The roles of transglutaminase 2 and FXIII-A in abdominal aortic aneurysm development

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Background: Abdominal aortic aneurysms (AAA) mainly affect men over the age of 65. Once they exceed 55 mm in diameter they may rupture with fatal consequences. AAA exhibit an inflammatory infiltrate, disrupted elastin and reduced elastin:collagen ratio. Studies have suggested that elastin is cross-linked to accessory proteins by transglutaminase 2 (TG2) to protect elastic fibres from proteolysis. The homologous enzyme, factor (F) XIII-A, may have a role in arterial remodelling and may act in concert with TG2.

Aims: To characterise the role of TG2 and FXIII-A in response to aneurysm induction.

Methods: TG2^{-/-}, FXIII-A^{-/-} and TG2^{-/-}, FXIII-A^{-/-} (DKO) mice underwent laparotomy at 8 weeks and 0.5M CaCl₂ was applied to the ventral aorta. Infra renal aortic diameter (AD) was measured by a blinded observer 6 weeks or 6 months post-surgery from calibrated images. Aneurysm development was defined as an increase of $\geq 50\%$ from baseline. Elastin and collagen were assayed at baseline using commercial kits and RT-PCR was performed on aortic mRNA samples

Results: Transcript levels for major structural proteins were similar between groups. The AD increased by 73% in CaCl₂-treated WT mice after 6 wk. TG2^{-/-} and FXIII-A^{-/-} mice showed similar increases in AD (83% and 75%) whereas the DKO mice increased by only 47% with fewer mice developing AAA (50% vs. 71%(WT), 80%(TG2^{-/-}), 71%(FXIII-A^{-/-}). Elastin:collagen ratios ($n = 8-12$) were 25 $\mu\text{g } \mu\text{g}^{-1}$ in WT, TG2^{-/-} and FXIII-A^{-/-} mice but were reduced in DKO mice (15 $\mu\text{g } \mu\text{g}^{-1}$), suggesting that increased collagen deposition was hindering dilatation. Aneurysm progression was monitored at 6 months in WT and TG2^{-/-} mice ($n = 7-10$). AD remained unchanged in WT mice (64% dilatation), but increased further in TG2^{-/-} mice (120%, $P = 0.035$)

Conclusion: TG2 does not affect the susceptibility of the AA to aneurysm induction but protects against later dilatation. Despite the lack of aneurysm development in DKO mice we hypothesise that this is not a protective phenomenon but may reflect deleterious changes in basal vessel structure

Disclosure of Interest: None declared.

PO097-WED

Factor XIA and thrombin generation are elevated in acute coronary syndrome and are predictors of recurrent cardiovascular events

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Background: In acute coronary syndrome (ACS) cardiac cell damage is preceded by thrombosis. Therefore, plasma coagulation markers may have additional diagnostic relevance in ACS.

Aims: By using novel coagulation assays this study aims to gain more insight into the relationship between the coagulation system and ACS.

Methods: We measured plasma thrombin generation, factor XIa and D-dimer levels in plasma from ACS patients ($n = 104$) and non-ACS patients ($n = 42$). Follow-up measurements were performed at 1 and 6 months. Associations between coagulation markers and recurrent cardiovascular events were calculated by logistic regression analysis.

Results: Thrombin generation was significantly enhanced in ACS patients compared to non-ACS patients: peak height 148 ± 53 vs. 122 ± 42 nM. There was a significantly diminished ETP reduction (32 vs. 41%) and increased contact activation (25 vs. 7%) in ACS compared to non-ACS patients. Furthermore, compared to non-ACS patients factor XIa and D-dimer levels were significantly elevated in ACS patients: 1.9 ± 1.1 vs. 1.4 ± 0.7 pM and 495 ($310-885$) vs. 380 ($235-540$) $\mu\text{g } \text{L}^{-1}$, respectively. Within the ACS spectrum, ST-elevation myocardial infarction patients had the highest prothrombotic profile. During the acute event, thrombin generation was significantly increased compared to 1 and 6 months afterwards: peak height 145 ± 52 vs. 100 ± 44 vs. 98 ± 33 nM. Both peak height and factor XIa levels on admission predicted recurrent cardiovascular events: OR 4.9 (95%CI $1.2-20.9$) and 4.5 ($1.1-18.9$).

Conclusion: ACS patients have an enhanced prothrombotic profile, demonstrated by an increased thrombin generation potential, factor XIa and D-dimer levels. This study is the first to demonstrate the positive association between factor XI activity, thrombin generation and recurrent cardiovascular events.

Disclosure of Interest: Nondeclared.

Atherosclerosis III

PO098-WED

Two peptide epitopes derived from human and mycobacterium, respectively, reduce atherosclerotic lesion in B6; 129S-LDLR (TM1HER) APOB (TM2SGY)/J mice

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Background: Atherogenesis involves cross talk between and shared pathways involved in adaptive and innate immunity. HSP60 has been shown to trigger innate and adaptive immune responses that initiate the earliest still reversible inflammatory stage of atherosclerosis.

Aims: To compare two HSP60 peptides derived from human and mycobacterium, respectively, for their ability to reduce atherosclerotic lesions through immunization in B6; 129S-Ldlr^{tm1Her} Apob^{tm2Sgy}/J mice.

Methods: Mice were immunized with two Keyhole limpet hemocyanin (KLH)-conjugated peptides derived from mycobacterial heat shock protein (HSP) 60 (AA253-268) designated as mHSP60²⁵³⁻²⁶⁸, human HSP60 (AA516-528) as hHSP60⁵¹⁶⁻⁵²⁸, respectively. Immunization of mice with these two peptides. Two weeks after the first immunization, mice were placed on a high-fat diet for 10 weeks.

Results: Immunization with these two peptides elicited high levels of antibodies against corresponding peptide in mice. Histological analyses demonstrated that the mice immunized with HSP60 showed equally significant reductions in atherosclerotic lesions (20.1% , $P < 0.001$; 25.9% ; $P < 0.001$) compared to control mice immunized with KLH alone. The effect on lesion size through immunization with these two peptides was supported by changes of sub-cell types including macrophage, cluster of differentiation (CD)4⁺ T cells, dendritic

cells and regulatory T cells as well as of levels of cytokines both in plasma and supernatant of splenocytes.

Conclusion: Two peptides, in spite of a low sequence homology (31%), have similar effects on significantly reducing early atherosclerotic lesions in the B6; 129S-Ldlr^{im1Her} Apob^{im2Sgy}/J mouse model of atherosclerosis. This approach offers attractive opportunities for design and development peptide-based vaccines against atherosclerosis.

Disclosure of Interest: None declared.

PO099-WED

The gut microbiota impacts plasmatic coagulation factors and augments arterial thrombus formation

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Background: Colonization of body surfaces by microbial communities and formation of the microbiota can be considered the most common and long-lasting persistent infection. Thrombus formation upon infection has been viewed as a response of the host to curtail infection, but some pathogens have evolved strategies to subvert the prothrombotic host responses to escape clearance.

Aims: To check if the enteric microbiota regulates coagulation factor synthesis and whether the absence of microbial colonization could affect experimental thrombus formation and hemostasis.

Methods: Germ-free mouse isolator technology combined with high-speed video fluorescence intravital microscopy. Western blot analysis, Coagulation factor IX and Factor X activity assays.

Results: In recent work, we identified the gut microbiota as a trigger of vascular remodeling via tissue factor (TF) signaling in the distal small intestine. We found that tail bleeding times were prolonged in germ-free mice (GF) compared with their conventionally-raised (CONV-R) counterparts. We reasoned that this could be due to changes in plasmatic coagulation factor levels or their activity. Western blot analyses indicated changes in plasma levels of Factor IX (FIX) and Factor X (FX) and activity of these factors in plasma was significantly higher in CONV-R mice compared with GF controls. To test if the gut microbiota impacts on experimental thrombus formation we subjected GF, CONV-D (ex-GF mice that were colonized for 14 days with a cecal microbiota harvested from a CONV-R donor mouse) and CONV-R mice to a ferric chloride injury model of the common carotid artery. Time to occlusion was significantly prolonged in GF compared with CONV-D and CONV-R mice.

Conclusion: Collectively, our results show that the intestinal microbiota increases plasmatic activities of FIX and FX and we propose that these microbiota-dependent changes could systemically affect hemostasis and experimental thrombus formation.

Disclosure of Interest: None declared.

PO100-WED

The role of clotting factor IX in the development of atherosclerosis

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Background: There is strong evidence for involvement of the coagulation system in the development and progression of atherosclerotic disease. Population studies initially demonstrated a decreased risk of acute myocardial infarction in hemophilic men compared with controls matched for age and gender; but these were not confirmed later

Aims: This study investigated the role of severe deficiency of factor IX, in the development of atherosclerosis, in C57BL/6 animals deficient in apolipoprotein E (APOE^{-/-}).

Methods: Groups APOE^{-/-} with (HB/APOE^{-/-}) or without (NL/APOE^{-/-}) FIX deficiency were compared by quantitative evaluation of lesions in the root and aortic extension, and histological characterization in the aortic root by immunofluorescence.

Results: Aortic lesions of animals HB/APOE^{-/-} were similar to the NL/APOE^{-/-} lesions after 8 weeks (1.8% vs. 1.6% respectively, $P = 0.74$) and 22 weeks of diet (8.1% vs. 7.2% respectively, $P = 0.64$). There was no difference in the quantification of atherosclerotic disease of aortic root among these groups after 8 weeks (0.047 vs. 0.053 mm² respectively, $P = 0.22$), and 22 weeks of diet (0.426 vs. 0.423 mm² respectively; $P = 0.64$). The patterns between groups in qualitative evaluation related to smooth muscle (α -actin) (5.59% vs. 7.62% respectively, $P = 0.17$) and macrophages (F4/80) (6.74% vs. 8.46%, respectively, $P = 0.30$) did not differ. However, representative analysis of aortic lesions showed decreased expression of interleukin IL-1 β , which corresponds to M1 macrophages, in HB/APOE^{-/-} compared to NL/APOE^{-/-}.

Conclusion: Despite the hypocoagulability of FIX deficiency, our results suggest that other factors are more important in the development of atherosclerosis, even in a model of high susceptibility to disease like APOE^{-/-}. The decrease of proinflammatory cells (M1 macrophages) in HB/APOE^{-/-} animals was not accompanied by a decrease in atherosclerotic lesions.

Disclosure of Interest: None declared.

PO101-WED

Phosphorylation of platelet acetyl-CoA carboxylase is increased in human and mice atherosclerosis and could be an indicator of persistent thrombin generation

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Background: Acetyl-CoA carboxylase (ACC) regulates fatty acids synthesis and oxidation. AMPK phosphorylates ACC on Ser79 and inhibits its activity. We recently showed that thrombin is the only platelet agonist leading to AMPK activation and subsequent ACC phosphorylation in human platelets.

Aims: We hypothesised that there is a relationship between platelet ACC phosphorylation and persistent thrombin generation *in vivo*. This work aims to assess whether ACC phosphorylation could be correlated to atherosclerosis, a thrombogenic situation.

Methods: Platelet ACC phosphorylation was analysed in patients referred to diagnostic coronary angiography and in a murine model of atherosclerosis (SR-BI/ApoE^{-/-}).

In humans, the demonstration and severity of the coronary artery disease was based on the coronary angiogram. In mice, the gravity of the disease was scored through histological assessment of lipid deposit and macrophages accumulation in the aortic root. Furthermore, mice platelet activation was evaluated *via* the detection of the phosphorylation of PKC substrates.

Results: Human patients with a proven coronary artery disease show a significant increase in platelet ACC phosphorylation as compared to control patients (patients who underwent coronary angiogram, but negative for coronary artery disease).

In mice, we confirm that atherosclerosis associated with an increase in platelet activation and we show a correlation between the level of platelet ACC phosphorylation and the severity of atherosclerosis.

Conclusion: Platelet ACC phosphorylation is associated to atherosclerosis and, accordingly to our hypothesis, could be an indicator of persistent thrombin generation. This could identify patients at high risk of subsequent thrombotic event and who could benefit for a long-term

antithrombin treatment in addition to the classical antiplatelet therapy.

Disclosure of Interest: None declared.

PO102-WED

Differential effects of leptin signaling in endothelial or smooth muscle cells on neointima formation following vascular injury in mice

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Background: Obesity is associated with elevated levels of the adipokine leptin due to hypothalamic leptin resistance. Leptin receptors are also expressed on vascular cells, and experimental studies have shown that leptin promotes neointima formation, whereas mice lacking leptin or its receptor are protected from restenosis.

Aims: To examine the role of EC- and SMC-mediated activation of LepR signaling during vascular remodeling and to determine whether the detrimental cardiovascular effects of obesity are due to elevated leptin levels or vascular resistance to protective effects of the adipokine.

Methods: Mice with loxP-flanked LepR alleles were mated with mice expressing Cre recombinase under control of the endothelial receptor tyrosine kinase (Tie2) or smooth muscle myosin heavy chain (SMMHC) promoter and fed tamoxifen-containing chow. Obesity was induced by 45% high fat diet. Neointima formation at the common carotid artery was examined 3 weeks after ferric chloride-induced vascular injury.

Results: Morphometric analysis revealed a significantly larger neointima and higher degree of lumen stenosis in End. LepR-KO mice, similar to diet-induced obese WT mice. Absence of LepR on SMCs did not affect neointima size, but resulted in a significantly greater media containing numerous morphologically de-differentiated cells. Analysis of primary ECs and SMCs from lean and obese mice or their conditioned medium confirmed differences in their migratory and paracrine capacities and the expression of factors mediating leptin resistance. Consistent with impaired vascular leptin signaling in obesity, leptin stimulation of endothelial progenitor cells isolated from obese, hyperleptinemic individuals enhanced murine neointima formation, in contrast to those isolated from lean subjects.

Conclusion: Our findings suggest that intact leptin signaling on ECs and SMCs differentially controls neointima formation and that obesity represents a state of vascular leptin resistance.

Disclosure of Interest: None declared.

PO103-WED

Binding of coagulation factor XIII to activated platelet subpopulations

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Background: Factor XIII (fXIII) is a transglutaminase zymogen circulating in blood and activated by thrombin. Its active form, fXIIIa, is known to bind activated platelets.

Aims: The aim of this study was to investigate interaction of fXIII with activated platelet subpopulations (phosphatidylserine –positive (PS⁺) and PS-negative (PS⁻) platelets).

Methods: Binding of fluorescently labeled fXIII to washed gel-filtered activated human platelets (either normal, or from patients with gray platelet syndrome or Glanzmann's thrombasthenia) was investigated using flow cytometry.

Results: fXIII bound at 300–500 molecules/cell under physiological conditions to platelets stimulated with thrombin or collagen-related peptide (CRP) known to induce coated platelet, but not to those stimulated with ADP that was not able to produce coated platelets or intact ones. However, binding of fXIII to ADP-stimulated platelets could be promoted by addition of fibrinogen. Binding to the PS⁺ platelets was four-fold better than to the PS⁻ ones. The binding was linear, reversible, without apparent saturation within the physiological fXIII concentration range and calcium-independent for both PS⁺ and PS⁻ platelets. Inhibitors of integrin α IIb β 3 notably decreased binding of fXIII to the PS⁻ platelets, but not to the PS⁺ ones. Binding of fXIII to thrombin-stimulated platelets from patients was decreased 3–4-fold for the PS⁺ platelets, and 4–8-fold for the PS⁻ platelets compared with healthy donors. With CRP, binding of fXIII to platelets from patients was reduced by an order of 7–9 for all subpopulations.

Conclusion: fXIII binds differently to PS⁺ and PS⁻ platelets. Binding involved alpha granule secretion and is fibrinogen-dependent. Binding of fibrinogen is in turn mediated by integrin α IIb β 3 in the PS⁻ platelets, and by the protein-rich protein 'coat' or 'cap' in the PS⁺ ones. This binding might be important for increasing fXIII local concentration in platelet thrombi.

Disclosure of Interest: None declared.

PO104-WED

Effect of dabigatran etexilate and warfarin on atherosclerotic plaque calcification in ApoE^{-/-} mice

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Background: Background. Vitamin K-antagonists (VKA) are the most frequently prescribed anticoagulant drugs to treat patients at risk of arterial or venous thrombosis. An undesired side effect of VKA is acceleration of vascular calcification in atherosclerotic lesions. Dabigatran etexilate is a novel oral anticoagulation qualified of replacing VKA treatment.

Aims: To compare effects of Dabigatran etexilate and Warfarin on plaque calcification in the apoE^{-/-} mouse model of atherosclerosis.

Methods: Female ApoE^{-/-} mice received Western type diet (WTD; control) or WTD supplemented with either Warfarin or Dabigatran etexilate for 6 or 20 weeks. Additionally, one group received 6 weeks WTD with Warfarin followed by 14 weeks of WTD with Dabigatran etexilate. Mice were sacrificed and aortas were analyzed on calcification by μ CT and immunohistochemistry.

Results: Warfarin treatment reduced atherosclerotic burden as suggested by decreased plaque sized after 6 weeks. In contrast, sustained Warfarin treatment exacerbates atherosclerosis demonstrated by increased plaque size compared to Dabigatran etexilate, and significantly increasing plaque calcification and elastin break numbers compared to both control and Dabigatran etexilate groups. Dabigatran etexilate treatment did not alter plaque size, calcification or elastin break numbers compared to control during 20 weeks of treatment. Switching diet from Warfarin to Dabigatran etexilate attenuated calcification, although not significantly.

Conclusion: Warfarin treatment attenuated plaque development in absence of atherosclerosis, whereas long-term Warfarin treatment aggravates atherogenesis by inducing severe calcification and increased

elastin breaks. Dabigatran etexilate treatment lacks the undesired side effects induced by long-term Warfarin treatment and improves plaque stability compared to Warfarin. Our results indicate that arterial vascular status is important for patients in need of oral anticoagulation and support personalized medicine.

Disclosure of Interest: None declared.

PO105-WED

Hyperglycaemic apolipoprotein-E deficient mice have accelerated atherosclerosis and aberrant vasa vasorum neovascularization

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Background: Vascular complications of diabetes mellitus have traditionally been separated into macro- and microvascular pathologies. The macrovascular complications of diabetes, such as accelerated atherosclerosis, contribute to disease mortality while microvascular disorders, including diabetic retinopathy, add to disease morbidity. Increasing evidence suggests that micro- and macrovascular complications may be linked.

Aims: Our objective is to determine if diabetes associated accelerated atherosclerosis is the result of hyperglycaemic effects on a microvascular bed, the vasa vasorum.

Methods: Hyperglycaemic streptozotocin-injected apolipoprotein-E deficient (ApoE^{-/-}) mice and normoglycaemic ApoE^{-/-} controls were utilized to examine the correlation between vasa vasorum density and progression of aortic atherosclerosis from 5 to 40 weeks of age.

Results: In normoglycaemic ApoE-deficient mice vasa vasorum neovascularization was directly proportional to the progression of atherosclerosis. Hyperglycaemic ApoE^{-/-} mice had significantly larger atherosclerotic lesion area and volume at the aortic sinus by 15 weeks of age ($P < 0.01$) but significantly lower vasa vasorum density compared to controls ($P < 0.05$). Hyperglycaemic mice at 15 weeks of age showed increases in vascular HIF-1 α expression ($P < 0.05$) and deficiency in VEGF expression suggesting changes in hypoxia-mediated angiogenesis may explain the initial lack of neovascularization. By 40 weeks of age hyperglycaemic ApoE^{-/-} mice had increased vasa vasorum neovascularization at the aortic sinus compared to the normoglycaemic ApoE^{-/-} mice ($P < 0.05$).

Conclusion: These findings suggest that the vasa vasorum is altered by hyperglycaemia. The microvascular changes seen in the vasa vasorum correlates with, and may influence, the development and progression of atherosclerosis in hyperglycaemic ApoE^{-/-} mice.

Disclosure of Interest: None declared.

Blood Coagulation Tests III

PO106-WED

The effect of homocysteine on the results of thrombodynamics test

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Background: To evaluate the blood level of homocysteine in pregnant women with complicated obstetric history (COH) and its impact on the results of the Thrombodynamics test

Aims: Of analysis of the Thrombodynamics test.

Methods: Collection of anamnestic and biological data was performed according to the rules of the international standard of ethics and qual-

ity of scientific research (GCP). The study included 28 pregnant women with COH observed in the Center of hemostasis and atherothrombosis. Preconception preparation of folic acid was performed. Diagnostics of blood coagulation was carried out on 'the Registrar of Thrombodynamics T2', which included such parameters as: delay the growth of the fibrin clot (Tlag, reference values of 0.8 - 1.5 min), the clot growth velocity (V, 20.5 - 30 $\mu\text{m min}^{-1}$), the density of the clot (D, 14,000 - 32,000 un.) and the presence of spontaneous clots (Tsp, are normally absent). Statistical analysis of data was performed using Stata/SE 11.2.

Results: The Thrombodynamics test results in the studied groups were within the reference values: Tlag - 0.8 min (0.8 - 1.1) and 1 min (0.8 - 1), V - 26.4 $\mu\text{m min}^{-1}$ (24.6 - 30.7) and 27.3 $\mu\text{m min}^{-1}$ (24.9 - 30.15), D - 27,445 un. (26,259 - 28,033) and 29,415 un. (28,996 - 31,561), for 1 and 2 groups, respectively. Tsp was observed in 2 pregnant women in 2 group, indicating hypercoagulation state. The median homocysteine levels in both groups were within the reference values: 5.93 $\mu\text{mol L}^{-1}$ (5.23 - 8.4) and 6.62 $\mu\text{mol L}^{-1}$ (4.02 - 8.18), respectively. Correlation analysis revealed no statistically significant relationship between level of homocysteine and results of the Thrombodynamics test. According to the results of genetic testing 15.4% of women were homozygous carriers of the MTHFR gene polymorphism (C677T), heterozygous carriers - 30.8%.

Conclusion: The presence of spontaneous clots in the third trimester may be associated with a physiological increase of the coagulation potential of blood during pregnancy and with max levels before childbirth.

Disclosure of Interest: None declared.

PO107-WED

Evaluation of a new homogeneous immunoassay for quantification of prothrombin fragment 1 + 2 (F1 + 2) in human plasma samples*

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Background: Prothrombin fragment 1 + 2 (F1 + 2) is generated during the conversion of prothrombin into active thrombin. The immunochemical quantification of F1 + 2 allows the determination of thrombin formation. F1 + 2 might have the clinical potential for assessing thrombotic risk and monitoring anticoagulation therapy. Here, we show first performance data of an automated immunoassay* for quantification of F1 + 2 based on LOCI® Advanced Chemiluminescence Technology. **

Aims: Characterization of the F1 + 2 assay.

Methods: LOCI technology is a homogeneous, chemiluminescent immunoassay approach that consists of a streptavidin-coated Sensi-bead particle, a Chemibead particle with immobilized antibody, and a second biotinylated antibody for immunometric assays. In the assay, the analyte forms bead-aggregated immune complexes via a primary neo-epitope-specific monoclonal antibody and an immune-complex-specific secondary monoclonal antibody, thereby generating a signal chain leading to a delayed, analyte-concentration-dependent luminescence emission.

Results: We have characterized the assay regarding limit of quantitation, stability, reproducibility, linearity, and potential interfering factors.

Conclusion: We conclude that use of LOCI technology has the potential to provide excellent sensitivity, precision, and dynamic range suitable to reliably measure F1 + 2 in human citrated plasma samples in a broad range without sample dilution. *Product under feasibility evaluation. Not available for sale, and its future availability cannot be guar-

anted. **LOCI and all associated marks are trademarks of Siemens Healthcare Diagnostics Inc. or its affiliates.

Disclosure of Interest: M. Ehm Employee of: Siemens Healthcare Diagnostics Products GmbH, D. Kraul Employee of: Siemens Healthcare Diagnostics Products GmbH, A. Stangl Employee of: Siemens Healthcare Diagnostics Products GmbH, M. Pfeiff Employee of: Siemens Healthcare Diagnostics Products GmbH, L. Feddersen Employee of: Siemens Healthcare Diagnostics Products GmbH, V. Smith Employee of: Siemens Healthcare Diagnostics Products GmbH, C. Duwe Employee of: Siemens Healthcare Diagnostics Products GmbH, U. Kuhn Employee of: Siemens Healthcare Diagnostics Products GmbH, O. Pohlack-Marsch Employee of: Siemens Healthcare Diagnostics Products GmbH, P. Vesper Employee of: Siemens Healthcare Diagnostics Products GmbH, E. Bousein-Mueller Employee of: Siemens Healthcare Diagnostics Products GmbH, N. Zander Employee of: Siemens Healthcare Diagnostics Products GmbH

PO108-WED

Identifying factor deficiencies by using the second derivative curve of APTT on an ACL-TOP coagulation analyzer

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Background: It was reported that the ACL-TOP coagulation analyzer can detect deficiencies of not only factor VIII (FVIII) and FIX, but also FXI and FXII by analyzing the waveform of the second derivative curve of activated partial thromboplastin time (APTT). However, it is not clear what level of each deficiency can be detected by waveform analyses.

Aims: We examined the usefulness of analyzing the waveform of the second derivative curve of APTT assayed by ACL-TOP for the identification of factor deficiency.

Methods: We made the mixtures of the normal pooled plasma and/or deficient plasma including FII, FV, FVII, FVIII, FIX, FX, FXI, FXII and prekallikrein. We performed APTT assay and the factor assay in these mixtures by using APTT-SP and SynthASil or Recombiplastin. To investigate the effect of von Willebrand factor (vWF) on the second derivative curve, we monitored the serial changes of waveform in an APTT assay after desmopressin infusion treatment for a patient with acquired von Willebrand disease (vWD).

Results: We detected biphasic abnormal curves in the deficient plasma of FV, FVIII, FIX and FXI. Furthermore, we detected a shoulder-type abnormal curve in plasma with 2–10% of the calculated levels of FVIII and FIX. In the cases of FV and FXI, the shoulder-type abnormal curve was found in 2–4% and 2%, respectively. We detected only a shoulder-type abnormal curve in the deficient plasma of FXII and prekallikrein. On the other hand, we did not detect any abnormal curves in the mixture of FII, FVII and FX. After 15 min of desmopressin infusion for the patient with acquired vWD, the level of vWF was increased to 54%. In accord with the increase of vWF level, the biphasic abnormal curve disappeared.

Conclusion: The waveform of the second derivative curve of APTT assayed by ACL-TOP might be a useful tool for detecting deficiencies of FV, FVIII, FIX, FXI, FXII, prekallikrein and vWF.

Disclosure of Interest: None declared.

PO109-WED

A rare combination: congenital factor vii deficiency with Chiari malformation

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Background: Congenital factor VII deficiency is the most common rare bleeding disorder.

Aims: We present a patient with congenital factor VII deficiency and congenital hydrocephalus who underwent a ventriculoperitoneal shunt operation and needed no prophylaxis after the procedure

Methods: A 20-day-old male neonate was born to a 28-year-old mother at 38 weeks of gestation. For his prenatal observation, there was unilateral hydrocephalus without intracranial hemorrhage. The blood coagulation tests were as follows: PT 50.6 s, APTT 35.8 s and INR 5.64. Factor VII assay was 2%. So, we diagnosed congenital factor VII deficiency. Cranial magnetic resonance visualization was performed and there was unilateral dilatation of the left ventricle due to congenital stenosis of foramen of Monro and minimal displacement of the septum pellucidum from the midline to the right side. All of these findings were components of Chiari malformation. Patient was decided to undergo a ventriculoperitoneal shunt operation. Recombinant factor VIIa was administered at a dose of 30 microgram kg⁻¹ every 2 h. There was bleeding neither during surgery nor postoperative period.

Results: A follow-up CT of the brain at 1 week showed slightly improved hydrocephalus and no bleeding. At present, the patient is on no prophylaxis and has no bleeding for the three months after the surgery

Conclusion: Congenital factor VII deficiency is characterized by a variable bleeding tendency.

Disclosure of Interest: None declared.

PO110-WED

Can comparing the value of the prothrombin time ratio and the activated partial thromboplastin time ratio help diagnose the cause of coagulopathy?

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Background: Prolongation of both prothrombin time (PT) and activated partial thromboplastin time (APTT) is found in many conditions. PT ratio and APTT ratio may vary in those conditions.

Aims: To compare the PT ratio (PT_r) and APTT ratio (APTT_r) in conditions with prolongation of both PT and APTT

Methods: Retrospective chart review of conditions with prolongation of both PT (PT_r ≥ 1.1) and APTT (APTT_r ≥ 1.1) was performed. Conditions comprised disseminated intravascular coagulation (DIC, *n* = 96), liver disease (LD, *n* = 200), vitamin K deficiency or warfarin use (VKD, *n* = 20), factor V inhibitor (FVI, *n* = 2), factor X inhibitor (FXI, *n* = 6). PT ratio divided by APTT ratio (PT_r/APTT_r) was calculated and compared among these conditions.

Results: Median (range) of PT_r in DIC was 1.41 s (1.11 to 3.82), LD 1.50 (1.10 to 5.94), VKD 2.72 (1.42 to 15.49), FVI 3.13 (1.89 and 4.37), FXI 3.52 (2.50 to 10.40). Median (range) of APTT_r in DIC was 1.42 s (1.13 to 3.27), LD 1.26 (1.13 to 4.68), VKD 1.65 (1.19 to 3.63), FVI 2.03 (2.00 and 2.06), FXI 1.92 (1.66 to 7.21). Mean±SD of PT_r/APTT_r in DIC was 1.01 ± 0.21, LD 1.22 ± 0.22, VKD 2.13 ± 0.88, FVI 1.53 (0.95 and 2.12), FXI 1.68 ± 0.32. PT_r/APTT_r in VKD was significantly higher than that in DIC (*P* < 0.001), LD (*P* < 0.001), and FXI (*P* = 0.014). PT_r/APTT_r in DIC was significantly lower than that in LD (*P* < 0.001) and FXI (*P* < 0.001). To differentiate between DIC and non-DIC group, using PT_r/APTT_r ≥ 1.06 to diagnose non-DIC group has the sensitivity of 81.6% (95% CI: 75.9 to 86.4) and specific-

ity of 64.6% (95% CI: 54.2 to 74.1). The receiver operator characteristic curve (ROC) demonstrated an area under the curve of 0.809 (95% CI: 0.756 to 0.863).

Conclusion: The value of PT ratio over APTT ratio was significantly higher in vitamin K deficiency/warfarin use than in DIC, liver disease, and factor X inhibitor. The value of PT ratio over APTT ratio in liver disease was significantly higher than that in DIC. The PTratio/APTT-ratio cut off point of < 1.06 was unlikely to be non-DIC cause.

Disclosure of Interest: None declared.

PO111-WED

Thrombin generation test for monitoring of conventional haemophilia treatment

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Background: The conventional factor replacement treatment of hemophilia patients needs the adequate laboratory support for the reliable monitoring of clotting factor activity. A major surgical procedure is supposed to be safely carried out under the factor cover of not < 80%. But the achievement of target activity level doesn't often reflect the global haemostatic balance.

Aims: The aim of our study was to evaluate the possibility of using the thrombin generation test (TGT) for the assessment of the clotting factor concentrates haemostatic efficacy.

Methods: Two patients with hemophilia A underwent the inguinal hernioplasty. Surgeries were performed under factor VIII concentrates (50 U kg⁻¹). The laboratory support included the monitoring of factor VIII activity (FVIII, %) and TGT. Thrombin generation was measured at Fluorocan Ascent[®] fluorometer using TF 1pM. The following parameters were analyzed: endogenous thrombin potential (ETP, nM*min) and the maximum concentration of thrombin (Peak, nM).

Results: Before the surgery the FVIII activity in patients was 2% and 3.6%, and ETP and Peak were also significantly reduced (233.9 nM*min and 11.6 nM, 207.8 nM*min and 11.6 nM, accordingly). These parameters of TGT in the control plasma were 763.3 nM*min and 66.8 nM, accordingly. In 30 min after the FVIII concentrate infusion the FVIII activity grew up to 67% and 74.8%, accordingly, i.e. the target activity level was not achieved. But ETP and Peak in both patients increased significantly and became 836.6 nM*min and 83.3 nM (109% and 124% compared with the control plasma), 786.9 nM*min and 90.7 nM (103% and 135%), accordingly. These findings confirm the sufficiency of the infusion rates in contrast to the FVIII activity value.

Conclusion: TGT may reflect the clinically relevant haemostatic activity of clotting factor concentrates; in addition, it may be useful in choosing the individual patients' dosing regimen.

Disclosure of Interest: None declared.

PO112-WED

Effect of recombinant lubricin on coagulation parameters in human blood

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Background: Lubricin (proteoglycan 4; PRG4) is a proteoglycan which acts at the joint/ boundary lubricant. Lubricin is present in synovial fluid and plays a major role in synovial homeostasis. A recombinant form of lubricin, rhPRG-4 is developed to treat a variety of inflammatory diseases, including osteoarthritis (Lubris Pharmaceutical, Boston, MA).

Aims: The purpose of this study is to determine the effect of rhPRG-4 on blood coagulation and platelet aggregation profiles utilizing standard experimental techniques.

Methods: rhPRG-4 was supplemented with citrated whole blood from normal human donors ($n = 10$) to obtain concentration range of 0 - 200 mg mL⁻¹. Activated clotting time (ACT), thrombelastographic analysis (TEG), and whole blood prothrombin time (PT), partial thromboplastin time (aPTT), and thrombin times (TT) were measured. Similarly, citrated plasma was supplemented with rhPRG-4 at 0-200 mg mL⁻¹ to determine PT, aPTT and TT. The effect of this agent was also studied on the generation of factors Xa and IIa using a fluorokinetic method. The effect of Lubricin on thrombin mediated fibrin formation was measured using a fibrinokinetic method. In addition, rhPRG-4 was also studied for its effect on platelet aggregation induced by various agonists.

Results: rhPRG-4 did not produce any effect on the whole blood clotting assays, such as the PT, aPTT and TT. Similarly, in the citrated plasma, none of the clotting assays were altered. rhPRG-4 did not produce any inhibition of thrombin generation in plasma. Furthermore it did not produce any inhibition or augmentation of agonist-induced platelet aggregation. Lubricin also did not produce any effect on fibrinokinetics profile. These studies suggest that rhPRG-4 does not modify whole blood and plasma coagulation profile and produce and more relation of platelet function.

Conclusion: These studies suggest that because of no potential alteration of the coagulation system, lubricin is not expected to modulate bleeding or thrombotic processes in clinical indications.

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PO113-WED

Characteristics of coagulation dynamics in acute phase of Kawasaki disease

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Background: Kawasaki disease (KD) is the systemic vasculitis involving coronary arteries mainly, resulting in complication with myocardial infarction due to coronary artery aneurysm. As one of mechanism(s), hypercoagulability in acute-phase (acute-) KD, based on change of individual clotting factor, is raised. However, little information on assessment of comprehensive coagulation function is known. A recently developed clot waveform analysis (CWA) and thrombin-plasmin generation assay (T/P-G) can assess global function by measuring parameters.

Aims: We investigated the clotting dynamics of acute-KD in both assays.

Methods: Forty-three patients of acute-KD with intravenous γ -globulin therapy (IVIG) at onset from 3 mo to 5 y.o. were enrolled. Blood

samples were collected, just before IVIG therapy (Pre), 1 week and 1 month after IVIG (1W and 1M). Cases who received anticoagulant drugs were excluded.

Results: In CWA, the clot time (CT) in Pre was prolonged, and both CT in 1W and 1M were more shortened than that in Pre. In contrast, the maximum coagulation velocity ($|min1|$) and acceleration ($|min2|$) in Pre showed ~2-fold higher levels than those in normal plasma, showing paradoxical response of CT. Both levels were decreased in 1W, and returned to normal levels in 1M. Behavior of $|min1|$ and $|min2|$ correlated with change of fibrinogen (Fbg), supportive of role of Fbg for hypercoagulability. In T/P-G, endogenous potentials of TG/PG in three points were greater than those in normal plasma, especially those in 1W were greater than those in Pre. For IVIG non-responders, $|min1|$ and $|min2|$ in 1W and 1M were greater than responders, supporting that non-responders remained hypercoagulability even in 1M.

Conclusion: Acute-KD shows the paradoxical coagulation dynamics with the developed hypercoagulability, despite the delayed initiation of coagulation. Acute-KD remains hypercoagulability even at 1 month after IVIG. Comprehensive functional assays should be useful to assess the pathological state of KD.

Disclosure of Interest: None declared.

PO114-WED

Ability of thromboelastography to distinguish the severity of phenotype in patients with severe hemophilia

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Background: Thromboelastography (TEG) provides a graphical representation of whole blood clot formation and fibrinolysis. In patients with hemophilia (PwH) the TEG curve has prolonged lag time and reduced amplitude. Different TEG profiles have been described in mild, moderate and severe PwH as well as in the presence of inhibitors (INH).

Aims: This study was aimed at investigating potential determinants of TEG variability in severe PwH.

Methods: Severe A and B PwH, of any age, with or without INH were included. Blood samples were collected in a non-bleeding state after a minimum wash-out period from treatment of 5 days. TEG was performed in whole citrated blood with the 4 channel ROTEM Gamma equipment. Coagulation Time (CT), Clot Formation Time (CFT), Maximum Clot Firmness (MCF) and α -Angle (α -A) were evaluated.

Results: TEG was performed in 62 PwH (median age 39 yrs): 41 (66%) had HA, 8 (13%) had INH, 32 (52%) had HCV and 18 (29%) HIV infection. F8/9 mutations were known in 58 (94%) PwH, being null in 26 (45%). TEG profile resulted more impaired in PwHA than in PwHB (CT, CFT, α -A: $P < 0.01$), in PwH with null mutations (CT, CFT, α -A: $P < 0.05$) and in those with viral infections (CFT, MCF, α -A: $P < 0.001$). Results were similar considering PwH with isolated HCV or HIV infection. TEG profiles were more impaired in INH than in non-INH PwH, however the difference did not reach statistical significance. No correlation between TEG parameters and INH titers was found. There was a significant correlation between TEG and platelet counts ($P < 0.01$) that were significantly lower in PwHA and in those with viral infections ($P < 0.05$).

Conclusion: This study showed a greater TEG impairment in severe PwH and null mutations than in PwH with less severe gene defects. Therefore, this global test is able to distinguish the severity of pheno-

type, however it is influenced by other biological factors as platelet count and co-morbidities as viral infections.

Disclosure of Interest: None declared.

PO115-WED

Whole blood coagulation analyses are dependent on the temperature they are performed at

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Background: Analysis of whole blood coagulation by thromboelastometry (ROTEM[®]) might depend on the temperature it is performed at, but similar data on platelet function tests are missing.

Aims: To examine whether thromboelastometric and platelet function tests differed when analyzed at 33 °C and 37 °C in hypothermic and normothermic patients.

Methods: We included 40 patients treated with hypothermia (33 ± 1 °C) after cardiac arrest. The first blood sample was obtained at hypothermia and the second at normothermia. Each blood sample was analyzed simultaneously at 33 °C and 37 °C using ROTEM[®] (EXTEM[®], INTEM[®], FIBTEM[®]) and platelet function tests (Multiplate[®] Analyzer) (COLtest[®], ADPtest[®], ASPItest[®], TRAPtest[®]).

Results: Comparing the ROTEM[®]-analyses at 33 °C and 37 °C we found a longer Clotting Time and a lower Maximum Velocity (all P -values < 0.008) at 33 °C independent of the patients being hypothermic (median 33.1 °C) or normothermic (median 37.5 °C). A longer Time to Maximum Velocity was found at 33 °C (all P -values < 0.002) except for EXTEM[®] during hypothermia ($P = 0.63$). No differences in the Maximum Clot Firmness (MCF) were found (all P -values > 0.082) except for a lower MCF at EXTEM[®] during hypothermia ($P = 0.027$). Comparing platelet aggregation performed at 33 °C and 37 °C during hypothermia we found no difference when using COLtest or TRAPtest (all P -values > 0.18), but the platelet aggregation was higher using the ASPItest ($P = 0.030$) and lower using the ADPtest ($P = 0.0001$). When comparing platelet aggregation carried out at 33 °C and 37 °C when the patients were normothermic we found no differences using ADPtest or TRAPtest (all P -values > 0.45), but a higher aggregation when performed at 37 °C using the COLtest and the ASPItest (all P -values < 0.0001).

Conclusion: Thromboelastometry showed a compromised coagulation when analyzed at 33 °C compared to 37 °C. In three out of four agonists the platelet aggregation was affected in different ways dependent on the patients being hypothermic or normothermic.

Disclosure of Interest: None declared.

PO116-WED

Correlation of peak thrombin concentrations with one-stage activity assay levels indicates that thrombin generation assays correlate with factor VIII activity in clinical hemophilia A plasma samples

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Background: Evaluating plasma hemostatic potential is critical for monitoring treatment and predicting bleeding risk in hemophilia patients. Factor assays, such as the one-stage activated partial thromboplastin time (aPTT) based assay, are standardized but offer an incomplete picture of

clotting. The thrombin generation assay (TGA) is a global hemostasis assay that allows for an expanded view of clotting and appears to correlate with clinical outcome. Here, we examine the relationship between TGA parameters and Factor VIII (FVIII) activity levels determined by aPTT in samples from a clinical study of Moroctocog Alfa AF-CC.

Aims: To determine whether TGA parameters are reflective of FVIII activity levels in patients who received FVIII replacement therapy.

Methods: Plasma sets from severe (FVIII:C $\leq 1\%$) Hemophilia A patients included a pre-dose plasma sample and plasma isolated 0.5, 8, 24, 28 and 32 h post dosing with Moroctocog Alfa AF-CC. Plasmas were analyzed in the aPTT assay on a STart 4 Hemostasis analyzer (Diagnostica Stago) and FVIII activities were extrapolated from a standard curve. For analysis in TGA, 80 μ L of plasma was added to 20 μ L of PPP-Reagent Low (Diagnostica Stago) prior to initiation with FluCa buffer (Diagnostica Stago) and analyzed in the Calibrated Automated Thrombogram assay (Diagnostica Stago). Linear regression analysis was performed on aPTT and TGA data sets. TGA parameters included lag time, endogenous thrombin potential, peak thrombin and time to achieve peak thrombin.

Results: Of the TGA parameters analyzed, peak thrombin correlated best with the measured aPTT values from the clinical plasma samples. There was considerable patient to patient variation and the observed correlation was more robust when analyzed by individual patient.

Conclusion: The TGA parameter of peak thrombin appears to best correlate with Factor VIII activity levels as measured by the one stage aPTT based activity assay.

Disclosure of Interest: S. Patel-Hett Employee of: Pfizer, J. Lee Employee of: Pfizer, S. Rakhe Employee of: Pfizer, S. Arkin Employee of: Pfizer, F. Huard Employee of: Pfizer, D. Pittman Employee of: Pfizer, J. Murphy Employee of: Pfizer, F. Hua Employee of: Pfizer

PO117-WED

Heparin interference in anti-XA assays for rivaroxaban and apixaban

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Background: Rivaroxaban and apixaban are target specific oral anticoagulants. Rivaroxaban (Xarelto[®]) is approved for stroke prevention in atrial fibrillation (SPAF), treatment of venous thromboembolism (VTE) and for VTE prevention after total hip and knee replacement. Apixaban (Eliquis[®]) is approved for SPAF. The anti-Xa activities of rivaroxaban and apixaban are higher than the activities of UFH and LMWH, therefore, specific rivaroxaban and apixaban calibrators and controls have been developed for the anti-Xa assay and are available for Research Use Only.

Aims: We examined and quantified the interference of therapeutic and supra-therapeutic levels of unfractionated and low molecular weight heparins (UFH, LMWH) on the rivaroxaban and apixaban specific anti-Xa assays.

Methods: Samples were collected from the clinical laboratory identified by prolonged aPTT and samples previously run for anti-Xa assay. Samples with anti-Xa levels above the therapeutic range (1.5–3.0 IU mL⁻¹) were purchased. A total of 88 samples were analyzed. Each sample was analyzed by aPTT and anti-Xa with three calibration curves: a hybrid curve for UFH and LMWH, rivaroxaban and apixaban. Assays were performed in singlicate and the values were compared by linear regression.

Results: Graphic representation of the APTT and the anti-Xa results demonstrated that there was no correlation using either of the three calibration curves. When heparin samples were assayed using either the rivaroxaban or apixaban calibration curve, heparin anti-Xa values ≤ 0.5 IU mL⁻¹ produced values ≤ 25 ng mL⁻¹, the lower level of quantification for the assays. Heparin samples ≥ 0.5 IU mL⁻¹ demonstrated a linear relationship to both the rivaroxaban and apixaban values with a correlation coefficient of 0.956 and 0.959, respectively.

Conclusion: We conclude that the overlap of UFH or LMWH therapy and the use of rivaroxaban or apixaban will result in anti-Xa results that are due to combined anti-Xa activity of the anticoagulants.

Disclosure of Interest: G. Cutsforth Employee of: Diagnostica Stago Inc, K. Finnegan: None declared.

PO118-WED

Use of lyophilized phospholipid vesicles in thrombodynamics assay

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Background: Addition of phospholipid vesicles is widely used in coagulation assays to replace platelets in their support of surface-dependent reactions. Reagents for thrombodynamics assay are provided in lyophilized form that requires only addition of plasma to the reaction tube. Use of phospholipid vesicles in the lyophilized reagents mixture could increase sensitivity of the assay and avoid sampling of small volume of phospholipid suspension.

Aims: The aim of this study was to develop a stable lyophilized reagent for thrombodynamics with the addition of phospholipid vesicles in order to increase accuracy and reproducibility of the measurements.

Methods: Phospholipid vesicles were prepared from pure phosphatidylserine and phosphatidylcholine by extrusion. Vesicles were lyophilized as a part of a standard reagent for thrombodynamics in a final concentration 4 μ M.

Results: We developed a new version of lyophilized reagent including phospholipid vesicles with long term shelf life (not < 12 months). Ten series of reagent including phospholipid vesicles were prepared in total. With the addition of phospholipids the stationary rate (Vs.) of fibrin clot growth in normal plasma was increased for approximately 27%. The coefficient of variation for the Vs. within these series were maximum 3% 7% with and without phospholipids respectively ($n \leq 18$ for each). The average CV (Vs.) for all the series with phospholipids were 2% ($n = 230$) and without phospholipids 4% ($n = 162$). Procoagulant activity of vesicles was decreased after lyophilization by approximately 5%. With the long storage at 4C for about 12 months the activity were decreased up to 10%, as estimated from Vs. value. **Conclusion:** The phospholipid vesicles in thrombodynamics increase the sensitivity of the assay by increasing Vs. The new lyophilized reagent provides high reproducibility of measurements and keeps the functional activity during long term storage.

Disclosure of Interest: N. Korotina Employee of: Hemacore LLC, L. Sokolova Employee of: Hemacore LLC, E. Ponomareva Employee of: Hemacore LLC, N. Dashkevich Employee of: HemaCore Labs LLC, F. Ataullakhanov Employee of: HemaCore Labs LLC

PO119-WED

Does antithrombotic therapy influence results obtained by thromboelastometry?Holck MH¹, Christensen TD^{2,3} and Hvas A-M^{1,3}¹Centre for Haemophilia and Thrombosis, Department of Clinical Biochemistry; ²Department of Cardiothoracic and Vascular Surgery, Aarhus University Hospital; ³Institute of Clinical Medicine, Aarhus University, Aarhus, Denmark

Background: During surgery or trauma antithrombotic treatment contributes to an increased risk of excessive bleeding if it has not been timely paused. Accordingly, it is essential to identify the impact of the medication on dynamic coagulation assays, which is often used in the acute situation. Our hypothesis was that antithrombotic therapy did not influence analysis performed by thromboelastometry (TEM) significantly.

Aims: To clarify whether the TEM-analysis was significantly influenced by antithrombotic therapy, or whether the effect of the medicine would be overlooked.

Methods: Patients ($N = 210$) in antithrombotic therapy were included; 70 patients receiving vitamin K antagonist (INR > 2.0), 70 patients receiving aspirin and 70 patients receiving aspirin and ADP-inhibitor. Whole blood coagulation was assessed by TEM using three standard assays INTEM, EXTEM and FIBTEM. Additionally APTT, INR, platelet count, haemoglobin, fibrinogen (functional), creatinine and CRP were analysed. S-Thromboxane B2 will be determined and used as compliance control for patients receiving aspirin. The Danish Ethics Committee (1-10-72-278-14) and the Danish Data Protective Agency approved the project. All participants signed an informed consent form and the project is following the Helsinki declarations.

Results: The vitamin K antagonist group had a prolonged clotting time for EXTEM (CT_{EXTEM}) with a median of 116 s (reference interval 38–74 s). CT_{EXTEM} was correlated to the INR level (Spearman $r = 0.53$; $P < 0.0001$). No other TEM-parameters were abnormal in the vitamin K antagonist group compared to reference intervals. Data for the aspirin and aspirin/ADP-inhibitor groups are still being collected and will be finished in March 2015. Thus, we will be able to present all results at the ISTH congress.

Conclusion: Preliminary results showed that the clot initiation time measured by TEM was prolonged in patients receiving vitamin K antagonists.

Disclosure of Interest: M. H. Holck: None declared, T. D. Christensen Speaker Bureau of: AstraZeneca, Boehringer Ingelheim, Pfizer, Takeda and Bristol-Myers Squibb, A.-M. Hvas: None declared.

PO120-WED

Screening method using two different activated partial thromboplastin time (APTT) reagents for specimens with prolonged APTTTakeo E¹, Suehisa E¹, Toku M¹, Kawasaki T² and Hidaka Y³¹Division of Laboratory for Clinical Investigation, Department of Medical Technology, Osaka University Hospital, Suita; ²Health and Medical Research Institute, The First Hospital of Welfare-Medical Association, Yao; ³Laboratory for Clinical Investigation, Osaka University Hospital, Suita, Japan

Background: Activated partial thromboplastin time (APTT) can be prolonged not only by a deficiency of coagulation factors but by various causes such as clotting factor antibodies and phospholipid antibodies.

Aims: By using two types of APTT reagent with different phospholipid compositions, we intended to investigate the possibility of differentiating coagulation factor deficiency and lupus anticoagulant (LA).

Methods: Two APTT reagents were used: APTT-SLA as the LA hypersensitivity reagent and APTT (FS) as the LA low-sensitivity reagent. Measurements were performed using a Coagrex-800 system. Results from the APTT-SLA were classified into normal and prolongation groups. These specimens were also measured with APTT (FS). We calculated the APTT clotting time ratio (APTT-CTR: APTT [FS] [s]/APTT-SLA [s]) from the measured values of the two APTT reagents as an index. Measurement of LA was by the dRVVT method (LA test 'Guradipoa'). A result of > 1.30 was considered positive with this method.

Results: The cut-off value of the APTT-CTR at the 5th percentile in the 235 normal group specimens was 0.834 (APTT-CTR: median, quartile deviation [QD]: 0.956, 0.048). In the 92 APTT-SLA prolongation group specimens, the APTT-CTR in 72 specimens were below the cut-off value (APTT-CTR: median, QD: 0.669, 0.077), and 70 of these 72 (97.2%) specimens were LA-positive. dRVVT-measured values in the remaining two specimens were 1.21 and 1.26, respectively. The LA-negative specimens above the cut-off value (APTT-CTR: median, QD: 0.929, 0.086) was 100% (20/20). Also, in 92 specimens (APTT-CTR: median, QD: 1.035, 0.076) with warfarin treatment (INR: 1.68–4.47), which were classified as being in the APTT-SLA prolongation group and LA negative, the APTT-CTR in 98% (90/92) were above the cut-off value. Specimens from hemophilia A patients ($n = 4$) did not have clotting factor antibodies: all had an APTT-CTR above the cut-off value.

Conclusion: The APTT-CTR may be useful to differentiate coagulation factor deficiency from LA.

Disclosure of Interest: None declared.

PO121-WED

Trombin generation; establishment of a reference interval and the effect of aspirinMogensen VB¹, Therkelsen MS¹, Nedergaard-Petersen S², Modrau IS³ and Hvas A-M¹¹Department of Clinical Biochemistry; ²Department of Cardiology; ³Department of Cardiothoracic Surgery, Aarhus University Hospital, Aarhus N, Denmark

Background: Analysis of thrombin generation is increasingly used for research purposes as a global measurement of hemostasis.

Aims: To establish a reference interval of thrombin generation and to investigate the effect of aspirin on thrombin generation in patients with coronary artery disease (CAD).

Methods: We included 90 healthy individuals, equally distributed in sex and five age groups; 21–30, 31–40, 41–50, 51–60, 61–65 years. To determine the effect of aspirin, we included 40 patients with CAD scheduled for coronary artery bypass. Blood was collected before surgery and both before and after treatment with a low dose of aspirin. Evaluation of thrombin generation by Calibrated Automated Thrombogram (CAT) was performed in citrated platelet poor plasma with addition of tissue factor (5 pM), phospholipids (4 μ M), calcium and a fluorogenic substrate to initiate coagulation. We recorded: Lag-time (min.) indicating time to thrombin formation start; Peak height (nM) indicating maximum thrombin generation; Endogenous thrombin potential (ETP, nM min⁻¹) indicating area under the thrombogram and time to peak (ttPeak, min.) indicating time to maximum peak. The study complied with the Declaration of Helsinki and was approved by the Central Denmark Region Committee on Biomedical Research Ethics (Project ID M-20100151) and informed consent was obtained.

Results: No difference was found between males and females (all $P > 0.06$). There was a significant, though small, difference between the 5 age groups as regards lagtime, peak height and time to peak (all $P < 0.04$), but ETP did not differ between age groups. We calculated a common reference interval: (mean [mean $\pm$ 1.96 SD]) lagtime 3.4

(2.6–4.3) min, peak height 201 (89–314) nM, ttPeak 7.2 (6.2–8.1) min, ETP 1281 (1011–1551) nM · min. In CAD patients thrombin generation was reduced during aspirin therapy ($P < 0.001$), except for ETP ($P = 0.16$)

Conclusion: A reference interval for thrombin generation was established. The results indicate reduced thrombin generation in CAD patients during aspirin.

Disclosure of Interest: None declared.

PO122-WED

A new assay for measurement of the *in-vivo* thrombin activity in comparison to the *in-vitro* thrombin generation potential in patients with hyper- and hypocoagulable state

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Background: The thrombin generation potential is an *in-vitro* measure for the capacity of an individual to generate thrombin and has been shown to reflect a hypo- or hypercoagulable state. Measurement of the *in-vivo* amount of thrombin activity may be of clinical significance.

Aims: We evaluated a new assay for *in-vivo* thrombin activity and compared it to the *in-vitro* thrombin generation potential in patients with hypocoagulability, such as in hemophilia ($n = 15$) or oral anticoagulation for atrial fibrillation (AF) ($n = 13$), and hypercoagulability represented by subjects with active cancer ($n = 21$).

Methods: Thrombin activity was measured with a commercially available oligonucleotide enzyme capture assay in argatroban-stabilized plasma samples. Thrombin generation potential was determined with a commercially available assay in citrated plasma.

Results: Thrombin activity was detected in 17 (34.7%) patients (median 0.4 mU mL⁻¹, 25.–75. percentile 0.28–1.35), and in 32 patients (65.3%) no thrombin activity was present. In cancer patients, thrombin activity was detected in 11 patients (range 0.14–5.00 mU mL⁻¹) and was particularly increased in 3 patients with vessel invasive tumors (1.2, 1.5 and 5.0 mU mL⁻¹). Thrombin activity was detected in 4 patients with hemophilia (range 0.29–1.75 mU mL⁻¹), all of whom had received a factor VIII infusion on the same day. In AF patients, thrombin activity was only measureable in 1 patient with peroneal palsy due to a hematoma (0.4 mU mL⁻¹) and 1 patient with recent history of ischemic stroke (1.5 mU mL⁻¹). Overall, peak thrombin generation potential was in median 116.6 nM (25.–75. percentile 26.3–259.7). Thrombin activity did not correlate with parameters of the thrombin generation potential (lag-time, peak thrombin, AUC).

Conclusion: In the majority of patients, no *in-vivo* thrombin activity was detected. Only patients in acute procoagulatory states had elevated thrombin activity, which was, however, unrelated to the *in-vitro* thrombin generation potential.

Disclosure of Interest: None declared.

PO123-WED

Influence of pre-analytical heparin on T-PA induced fibrinolysis determined by thromboelastography despite the use of discard tubes

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Background: Despite the consequent use of discard tubes before sampling, contamination of blood samples with heparin from the heparin lock of central venous catheters, is often seen in daily practice. In thromboelastometric (ROTEM®) analysis, heparin effect is seen in the INTEM but not in the EXTEM. t-PA ROTEM can be used to evaluate clot lysis. Since it contains tissue factor to activate the extrinsic pathway, we expect no effect of heparin on this test.

Aims: To evaluate the pre-analytical effect of heparin on t-PA ROTEM and evaluate polybrene as a heparin neutralizer.

Methods: Blood was drawn from healthy volunteers, and *in vitro*, heparin solution was added in different concentrations. To neutralize the effect of heparin, polybrene solution was added in different concentrations. aPTT, thrombin time and t-PA ROTEM were performed in all samples.

Results: Compared to control samples, a heparin concentration of 0.1 IU mL⁻¹ resulted in a slightly prolonged aPTT (33.7 s vs. 27.3 s) and thrombin time (23.9 s vs. 16.2 s). There was a positive correlation between heparin concentration and aPTT (r_s 1.00), and heparin concentration and thrombin time (r_s 0.98, 95%CI: 0.70–0.99). In plasma, 2.5 µg mL⁻¹ polybrene was able to neutralize the effects of heparin (up to 0.5 IU mL⁻¹) on the aPTT and thrombin time, but an excess of polybrene in plasma prolonged the aPTT. Unexpectedly, heparin effect was seen on t-PA ROTEM results. Adding heparin resulted in a decrease in MCF and clot lysis speed. 2.5 µg mL⁻¹ polybrene was not able to normalize these results. Furthermore, when only polybrene was added to the blood samples, this also resulted in abnormal MCF and clot lysis speed.

Conclusion: The heparin effect on aPTT and thrombin time can be neutralized by adding polybrene. Heparin has a clear effect on the t-PA ROTEM in healthy individuals and polybrene does not fully neutralize this effect. Evenmore, polybrene itself has an effect on t-PA ROTEM. Polybrene is not suitable for neutralizing the heparin effect on the t-PA ROTEM.

Disclosure of Interest: None declared.

PO124-WED

Effect of fresh frozen plasma in critically ill patients

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Background: Critically ill patients receive fresh frozen plasma (FFP) because it potentially reduces the risk of bleeding. Transfusion of FFP often relies on abnormal conventional coagulation tests, but the evidence for this strategy is limited.

Aims: To investigate the biochemical effect of FFP transfusion on conventional coagulation tests and thromboelastometry (TEM) and hereby clarify if TEM improves the decision-making prior to FFP transfusion.

Methods: We aim to include 50 patients at the Department of Intensive Care Medicine, Aarhus University Hospital if the patient receives a minimum of two units FFP once during admission. Blood samples are obtained prior to FFP transfusion, and 1 h after the last unit has been

transfused. Besides TEM, we perform conventional coagulation tests. The study complies with the Declaration of Helsinki and Danish law of ethics.

Results: For now, 24 patients are included. In the majority of patients, parameters of conventional coagulation tests were abnormal both before and after FFP transfusion (Prothrombin time (normal > 0.80): median 0.29; $n_{\text{abnormal}}=23$ before vs. median 0.34; $n_{\text{abnormal}}=22$ after. Activated partial thromboplastin time (normal < 38 s): median 41 s; $n_{\text{abnormal}}=19$ before vs. median 41 s; $n_{\text{abnormal}}=17$ after. Fibrinogen (normal > 5.5 micromole L⁻¹): median 8.8; $n_{\text{abnormal}}=8$ before vs. median 8.8; $n_{\text{abnormal}}=7$ after). In a minor part of the patients, the TEM-analysis revealed parameters outside of the reference interval both before and after transfusion (EXTEM clotting time: $n = 9$ before vs. $n = 6$ after, INTEM clotting time: $n = 12$ vs. $n = 11$ after and FIB-TEM maximum clot firmness: $n = 5$ before vs. $n = 3$ after).

Conclusion: Preliminary results show that most patients have abnormal conventional coagulations tests before FFP transfusion in contrast to TEM showing a normo-coagulable state in the majority of patients. The biochemical effect of FFP was minimal. The results suggest that the decision-making before FFP should be revised.

Disclosure of Interest: None declared.

PO125-WED

A DRVVT-based assay to estimate the intensity of anticoagulation in patients treated with NOACs

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Background: The dilute Russel Viper Venom Time (dRVVT) has been well correlated with dabigatran and rivaroxaban plasma concentrations in clinical samples. However, drawbacks have been highlighted in previous studies such as the inter-individual variability and the need of reconstitution of the reagents. This has stimulated improvement of the procedure and the methodology.

Aims: To compare the results of an optimized dRVVT-based NOAC assay (DRVV-NOAC) with estimated plasma concentrations of apixaban, dabigatran and rivaroxaban in clinical samples, and to evaluate the effect of mixing test samples with normal pooled plasma (NPP) to reduce inter-individual variability.

Methods: Seven apixaban, 29 dabigatran and 82 rivaroxaban samples from patients were included. Plasma concentrations were estimated with the Hemoclot Thrombin Inhibitor[®] for dabigatran samples and the Biophen Direct FXa Inhibitor[®] for rivaroxaban and apixaban samples. DRVV-NOAC was performed with an original ready-to-use reagent (Haematex ResearchTM); plasma samples were tested neat and in a 1:1 mix with NPP. Homemade and commercial calibrators were used to assess the matrix effect. The calibration also aimed at providing results in ng mL⁻¹.

Results: The plasma range concentration was from 125 to 441 ng mL⁻¹ for apixaban, 0 to 620 ng mL⁻¹ for dabigatran and 0 to 554 ng mL⁻¹ for rivaroxaban. Mixed calibrated DRVV-NOAC test correlated better than the non-mixed procedure with the estimated plasma concentrations ($r^2 = 0.99$ vs. 0.98, 0.95 vs. 0.93 and 0.87 vs. 0.68 for apixaban, dabigatran and rivaroxaban respectively). The Bland-Altman analyses of the mixed procedure showed important differences of the mean deviations according to the calibrators used (from -61 to 89 ng mL⁻¹ for apixaban, -21 to 114 ng mL⁻¹ for dabigatran and -63 to 33 ng mL⁻¹ for rivaroxaban).

Conclusion: DRVV-NOAC with mixing procedure could be used to screen the relative intensity of anticoagulation of NOACs. Mixing

sample with NPP before testing improves correlation parameters and Bland-Altman analyses.

Disclosure of Interest: None declared.

PO126-WED

Pre-analytical sample check in haemostasis: evaluation of the new coagulometer ACL Top 750 CTS system

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Background: An important goal for the hemostasis laboratory is to identify any possible interference related to sample quality and/or to clinical conditions that can cause functional interferences on test results.

Aims: We have compared a new analyzer, ACL TOP 750 CTS with the ACL TOP 700 CTS (Instrumentation Laboratory, Bedford, MA). This new system performs a pre-analytical check that detects incorrect filling volumes of the tube, recognizes possible obstruction during the aspiration (clog-clot detection) and identifies optical interferences (hemolysis, bilirubin and lipemia) by means of a new multiwavelength optical module.

Methods: As suggested by CLSI Guidelines, we ran normal and abnormal control materials 20 times per day for 5 days on four coagulation parameters (PT, APTT, AT and DD) to cover clotting, chromogenic and immunoturbidimetric methods. Means, SD and CV% were calculated. The turn-around time (TAT) of the two analyzers were compared for the routine profiles (single or multiple test). On ACL TOP 750 CTS we analyzed the samples that would have been rejected according to our preanalytical guidelines (~2.2% of our daily workload).

Results: We found overlapping mean values and comparable CV% for all the parameters/levels analyzed. No negative impact on analytical TAT was observed in any condition (routine samples, controls, single or multiple test). According to our guidelines, that include visual inspection of the sample, the new ACL TOP 750 CTS recognized properly tubes with insufficient filling volume, samples with micro-clots not initially identified; the new system flagged ~30% on previously selected group of samples rejected as hemolysed. All the lipemic samples were identified by the system. No icterus samples were found during our observation.

Conclusion: The ACL TOP 750 CTS has demonstrated the ability to identify potential preanalytical errors by means of specific algorithms without throughput reduction. The automated and standardized checks of all the pre-analytical interferences can reduce TAT.

Disclosure of Interest: None declared.

PO127-WED

Thrombin generation in patients undergoing major orthopaedic surgery

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Background: Patients undergoing major orthopaedic surgery are prone to develop both thrombotic and bleeding complications. Traditional coagulation parameters are not useful tools to estimate either the thrombotic or the bleeding risk. Thrombin generation (TG) provides greater information as the parameters of TG: describe the time until thrombin

burst (lagtime in min), peak thrombin (nM) and the total amount of thrombin generated (endogenous thrombin potential (ETP, nM*min).

Aims: To evaluate thrombin generation (TG) in a group of consecutive patients undergoing major orthopaedic surgery.

Methods: TG as well as classical clotting markers was evaluated in twenty patients (M/F 8/12, age range 48–90) undergoing major orthopaedic surgery. The samples were collected immediately pre-surgery (T0-pre), immediately post conclusion of surgery (T0-post), one day post surgery (T1) and 5 ± 2 days post surgery (T2). TG was determined in Platelet Poor Plasma using the CAT method (Diagnostica Stago, Asnieres, France).

Results: ETP (mean ± SD) at T1 (1205.5 ± 184.4 nM*min) was significantly lower than at T0-pre (909.9 ± 217.4 nM*min, $P < 0.001$). Peak thrombin (mean ± SD) at T1 (197.7 ± 63.8 nM) was significantly lower than at T0-pre (254.0 ± 61.9 nM, $P < 0.01$). TG parameters at T2 returned with values similar to that observed preoperatively. No significant difference of traditional coagulation parameters was observed at any time-points considered in our study.

Conclusion: The changes observed in our study, as far as the TG profile was concerned, may contribute to the thrombotic and/or bleeding risk that occurs in the perioperative period after major orthopaedic surgery. Future prospective studies aimed at correlating haemostatic changes with clinical perioperative outcomes are warranted.

Disclosure of Interest: L. Spiezia: None declared, F. Vasques: None declared, A. Behr: None declared, F. Dalla Valle: None declared, B. Woodhams Shareholder of: Haemacon Ltd, S. Gavasso: None declared, P. Simioni: None declared.

PO128-WED

Evaluation of the automated coagulation analyser Sysmex® CS-5100 (SIEMENS)

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Background: With a throughput of up to 400 tests per hour, the Sysmex® CS-5100 (SIEMENS) is a fully-automated random-access multi-parameter coagulation analyzer equipped with a photo-optical clot-detection unit, a cap-piercing system and a pre-analytical check screens for interfering substances such as bilirubin, lipids and haemolysis (HIL system). It is designed to perform clotting, chromogenic, immunoturbidimetric and platelet aggregation assays with continuous loading capabilities for samples, reagents and disposables.

Aims: The aim of the study was to evaluate its performance.

Methods: The instrument was evaluated in the coagulation laboratory of the Pitié-Salpêtrière Hospital with respect to its technical features in the determination of routine coagulation (prothrombin time, activated partial thromboplastin time, fibrinogen and single coagulation factor levels), chromogenic (anti-activated factor X, antithrombin), immunoassay and platelet aggregation method (D-Dimers, von Willebrand factor antigen and activity, RCo).

Results: Using fresh and lyophilized plasma samples, the intra-assay and inter-assay coefficients of variation (CV) were below 6% for most parameters both in the normal and in the pathological range (exceptions: intra-assay for severe factor VIII deficiency (CV = 7.45%) and for RCo (CV = 7.26% et 7.70%); and inter-assay for anti-activated factor X (CV = 8.83%) and RCo (9.34% et 11.13%). The measured lower limits of linearity for factor VIII and factor V were satisfactory. No sample or reagent carryover was detected in the conditions of the study. The results of the different coagulation assays obtained on the CS-5100 were well correlated with those obtained on the STAR analyzer.

Conclusion: Using the the Sysmex® CS-5100 analyzer, routine hemostasis testing can be performed with satisfactory precision and the same applied to more specialized and specific tests such as single factor

activity or antigen concentration. This automate has the advantage of being connectable to an automation chain.

Disclosure of Interest: None declared.

PO129-WED

Impact of assay, product, and patient variability on precision dosing for factor VIII prophylaxis

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Background: Personalizing prophylaxis using pharmacokinetic (PK) data prevents joint and other bleeding in patients with hemophilia A.¹

Aims: We theorized that the factor (F) VIII assay used, FVIII product selected, and interpatient differences impact PK assessment and precision prophylactic dosing.

Methods: A comprehensive literature search for articles published from 2004 to 2014 was performed. A total of 363 English language articles were found, of which 99 were used to identify the variables associated with these 3 domains.

Results: The variables identified were:

- 1 Type of FVIII assay (1-stage clotting or chromogenic) and its components, including reagents, diluents, buffers, standard, activator, and FVIII deficient plasma and its von Willebrand factor concentration, which are used to diagnose hemophilia, determine its severity, and monitor post-infusion samples,
- 2 Differences among FVIII products with regard to labeled potency, lot-to-lot consistency and quality, *in vivo* metabolism, modifications to the FVIII protein, and amount of free polyethylene glycol that affect the magnitude and duration of the prophylactic effect.
- 3 Patient-to-patient variability, such as presence of target joints, level of physical activity, adherence, thrombin-generating ability, antibody levels, and FVIII modification, that influence bleeding risk and response to prophylaxis.

Conclusion: FVIII assays and products along with interpatient differences impact PK and the ability to fine-tune FVIII prophylaxis. Regimens that are personalized using all available information are better tailored to the individual and may be more cost-effective and widely available, thereby improving long-term outcomes, including joint health, for patients with hemophilia A.

Reference: 1. Valentino LA, Mamonov V, Hellmann A, et al. A randomized comparison of two prophylaxis regimens and a paired comparison of on-demand and prophylaxis treatments in hemophilia A management. *J Thromb Haemost.* 2012; 10 (3):359–367.

Disclosure of Interest: L. Valentino Employee of: Baxter Healthcare Corporation, P. Turecek Employee of: Baxter Healthcare Corporation, K. Mann: None declared.

PO130-WED

Evaluation of waveform analysis on the destiny max coagulation analyser

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Background: Coagulation dynamic and reporting of end-point clotting times reflects only part of the process ignoring kinetics of clot formation or its quality. Generation of clot waveforms from optical prothrombin time (PT) or activated partial thrombin time (APTT)

based assays provide characteristic patterns that can be informative in specific clinical conditions.

Aims: Evaluating and identifying specific waveform patterns and associated mathematical curve descriptors for PT and APTT based assays in normal subjects and patients with congenital or acquired coagulation abnormalities using the new Destiny Max clot waveform analysis (CWA) software.

Methods: Assays were performed on Destiny Max coagulation analyser (Stago UK). PT and APTT were performed using optical clot detected and TriniCLOT PT, TriniCLOT aPTT and TriniCHECK controls. CWA was performed on citrated plasma samples from normal, warfarin therapy and Haemophilia A patients, with measurements calculated for maximum clot velocity (Min 1), maximum clot acceleration (Min 2) and maximum deceleration (Max 2).

Results: For warfarin therapy patients there was a statistically significantly with PT for Min 1, $P = 0.026$ and Min Max, $P = 0.029$ compared to normal plasma. For Haemophilia A patients there was a statistically significantly with APTT for Min 1, $P = 0.004$, Min 2, $P = <0.001$ and Min Max, $P = 0.004$ and with von Willebrands Disease there was a statistically significantly with APTT for Min 1, $P = 0.024$, Min 2, $P = 0.033$ and Min Max, $P = 0.020$.

Conclusion: PT and APTT based assays in conjunction with the new CWA software provide ability to assess global haemostasis function. Assessment of CWA parameters against an established normal range particularly in patients with congenital or acquired bleeding disorders provides an additional diagnostic tool to characterised individual patient bleeding phenotypes.

Disclosure of Interest: None declared.

PO131-WED

Four-factor prothrombin complex concentrate shows anticoagulant effect when used for *in vitro* experiments

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Background: An increasing number of publications test the *in vitro* effect of prothrombin complex concentrates for reversal of new oral anticoagulants. Four-factor prothrombin complex concentrate (4F-PCC) containing heparin has shown less favorable results compared to other PCCs.

Aims: To clarify whether the content of heparin in 4F-PCC influences the *in vitro* experiments.

Methods: Blood samples were obtained from three healthy women. Titration experiments were performed by thromboelastometry using a low tissue factor assay (final dilution 1:100,000). Firstly, we added increasing concentrations of 4F-PCC (final concentration: 0–43 IU kg⁻¹). Secondly, increasing concentrations of protamine sulfate (final concentration 0–0.32 mg mL⁻¹) were added to a fixed concentration of 4F-PCC (final concentration 30 IU kg⁻¹). The study complied with the Declaration of Helsinki.

Results: Thromboelastometric analyses indicated compromised hemostasis after addition of 4F-PCC. This was demonstrated by a dose-response reduced clot initiation indicated by increased mean clotting time (464 ± 25 to 1428 ± 455 s, $P = 0.001$) and reduced clot propagation indicated by increased mean clot formation time (133 ± 37 to 805 ± 146 s, $P < 0.0001$), reduced maximum velocity (10.7 ± 2.8 to 2.2 ± 0.3 mm min⁻¹, $P = 0.003$) and increased mean time to maximum velocity (553 ± 38 to 2043 ± 637 s, $P = 0.0003$) and finally reduced clot strength indicated by reduced mean maximum clot firmness (58 ± 6 to 35 ± 5 mm, $P = 0.02$). A concentration of 0.04 mg mL⁻¹ protamine sulfate reversed 5–12.5 IU heparin mL⁻¹

4F-PCC. Addition of higher concentrations of protamine sulfate revealed an anticoagulant effect.

Conclusion: This *in vitro* experiment showed that the heparin content in 4F-PCC compromised hemostasis considerably and thereby might provide misleading results when 4F-PCC are used for *in vitro* experiments.

Disclosure of Interest: None declared.

PO132-WED

Evaluation of a new, innovative, intermediate throughput coagulation analyser

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Background: We assessed the performance of a new coagulation analyser (CS-2500, Sysmex Corporation, Japan) capable of performing platelet aggregation studies in addition to routine clotting, chromogenic and immunoturbidometric assays.

Methods: Performance was compared to the CS-2100i for clotting (prothrombin time, activated partial thromboplastin time, Clauss Fibrinogen, Thrombin Time, Dabigatran), chromogenic (Antithrombin, Rivaroxaban) and immunoturbidometric (D-Dimer) test systems. All reagents were supplied by Siemens Diagnostic Healthcare (Marburg, Germany) except for Dabigatran and Rivaroxaban assays which were supplied by Hyphen Biomed (Paris, France). Commercial calibrants and control samples were assessed as well as samples from healthy normal subjects ($n = 30$) and a wide variety of different patients ($n = 95$) (selected to represent the common types of abnormality that might be encountered in the clinical laboratory). Calibration curves were tested once on the CS-2100i and on five consecutive days on the CS-2500. Each control sample was tested 10 times to obtain imprecision data.

Results: The CS-2500 showed good linearity of standard curves in the assays and low inter-assay imprecision in all tests using commercial normal (CV=0.3–4.1%) and pathological (CV = 0.5–5.0%) control samples. Using samples from a range of patients and normal subjects, good correlation was observed between the CS-2500 and CS-2100i in each of the test systems measured ($R^2 = 0.98$ – 1.00), with no clinically significant misclassification, and data points scattered closely around the line of identity. There was no difference between analysers due to interfering substances. Sample analysis was 180 PT and 62 PT+APTT samples per hour on the CS-2500, compared to 180 PT and 53 PT+APTT on the CS-2100i.

Conclusion: Our results demonstrate that using the CS-2500 analyser, routine and specialised coagulation testing can be performed with satisfactory levels of imprecision and showing good correlation with the CS-2100i.

Disclosure of Interest: None declared.

PO133-WED

Haemophilia A carrier detection in sudanese families: DNA linkage analysis approaches

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Background: Haemophilia A is the most common X-linked inherited bleeding disorder caused by a deficiency in the activity of coagulation factor VIII, with an incidence of 1 in 5000 male births. Genetic diagnosis of Haemophilia A is the most accurate method available for carrier

detection. Direct mutation detection for haemophilia A is difficult and expensive, accordingly genetic testing for carrier detection has relied upon indirect linkage studies.

Aims: To investigate the usefulness of FVIII intron 18 *BclI* RFLP, intron 13 and intron 22 CA repeats linkage analysis for carrier detection in Sudanese families.

Methods: This was a prospective, cross sectional, analytical and community-based study. Following written informed consent 20 families with at least one subject affected with Haemophilia A, and 30 unrelated normal females as control group were enrolled. PCR and restriction enzyme analysis were used to study the polymorphism in *BclI*. Intron 13 and intron 22 CA repeats were analyzed using fluorescent PCR followed by capillary electrophoresis.

Results: The incidence of *BclI* (+) allele was 78%, 39.5% and 33% in patients, female relatives and control group respectively. Expected heterozygosity for *BclI* was 0.48 in female relatives compared with 0.46 in the female control group. However, observed heterozygosity was found to be 0.54 in female relatives compared to 0.66 in the control group. The defective X chromosome was tracked in 13/20 (65%) mothers, hence 65% of the studied families were found to be informative using *BclI*-RFLP. Intron 13CA repeats were studied in 9 families. The defective X chromosome could be tracked down in 4/9 (44.4%) mothers. Intron 22 CA repeats were studied in 11 families and all families were uninformative.

Conclusion: PCR-RFLP using *BclI* is informative in carrier detection of Haemophilia A in the Sudanese population. *BclI* is more informative compared to both Introns 13 and Intron 22 CA repeats.

Disclosure of Interest: None declared.

PO134-WED

Impact of silver nanoparticles on hemolysis, platelet function, complement system and coagulation

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Background: Silver nanoparticles (Ag NPs) are increasingly used in biomedical applications because of their large antimicrobial spectrum. Data in the literature on the ability of Ag NPs to perform their desired function without eliciting undesirable effects on blood elements are very limited and contradictory.

Aims: The objectives of this study were to assess the impact of Ag NPs on erythrocyte integrity, platelet function, complement system and blood coagulation.

Methods: Erythrocyte integrity was assessed by spectrophotometric measurement of haemoglobin release. Platelet adhesion and aggregation was determined by light transmission aggregometry and scanning electron microscopy. The calibrated thrombin generation test was used to study the impact on coagulation cascade. Activation products of complement cascade were measured by ELISA.

Results: We demonstrated that Ag NPs induced haemolysis. They also increased platelet adhesion without having any impact on platelet aggregation. Ag NPs had a procoagulant potential. No effect on the complement cascade was observed. Bringing all data from these tests together, the no observed effect concentration was 5 µg mL⁻¹.

Conclusion: Ag NPs have a hemolytic potential, a procoagulant activity and a slight antiaggregant potential. These preliminary *ex vivo* results suggest that the hemocompatibility of Ag nanomaterials should be carefully assessed, especially those used for medical applications.

Disclosure of Interest: None declared.

PO135-WED

Determination of insufficient anticoagulant effect in patients with atrial fibrillation using thrombodynamics assay

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Background: Stroke and bleeding are still frequent complications in patients with atrial fibrillation under warfarin therapy despite INR control.

Aims: We aimed to determine the group of patients with insufficient anticoagulant therapy.

Methods: Two hundred and six patients with AF under warfarin for at least 30 days were enrolled in the study. INR, D-dimer level and thrombodynamics were compared. In thrombodynamics (TDx) assay the velocity of clot growth (V) is measured (reference values are 20–30 µm min⁻¹).

Results: We used D-dimer level to estimate whether increased fibrin formation took place previously to blood sampling. Patients were divided into two groups: with elevated D-dimer level (D-d > 250 ng mL⁻¹, 23 patients, 11%) and with normal D-dimer level. Age, sex and CHA2DS2-VASc score didn't differ between the groups. We found no significant difference in INR between groups with increased and normal D-dimer level (medians 1.9 and 2.0). V was significantly higher in the group with increased D-dimer level (medians 22.3 and 19.1 µm min⁻¹, *P* < 0.05). ROC-analysis was used to evaluate the ability of INR and V to predict D-dimer increase. INR showed no ability to separate the groups (AUC=0.518). V showed better ability to separate the groups (AUC=0.65) with high negative predictive value (93.7%) with *V* < 19.7 µm min⁻¹ (cut-off). Using found cut-off value for V we estimated the amount of patients with contradiction between INR and V data. 34 patients (16.5%) showed sufficient anticoagulant effect by TDx (*V* < 19.7 µm min⁻¹) despite INR showing poor effect (INR < 2); none of them had increased D-dimer level. 20 patients (9.7%) showed poor anticoagulant effect by TDx (*V* > 19.7 µm min⁻¹) despite INR showing sufficient effect (INR > 2); 6 of them had increased D-dimer level indicating the lack of anticoagulant effect.

Conclusion: We could conclude that thrombodynamics clot growth rate V has a high (93.7%) negative predictive value for increased D-dimer level in patients with atrial fibrillation under warfarin therapy.

Disclosure of Interest: N. Topalov Employee of: HemaCore LLC, I. Zotova: None declared, D. Zateyshchikov: None declared, F. Ataullakhanov Employee of: HemaCore LLC, I. Serebriyskiy Employee of: HemaCore LLC

PO136-WED

New liquid recombinant thromboplastin (DG PT RecombiLIQ, diagnostic grifols) suitable for routine use

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Background: Prothrombin time reagents should have manual ISI towards the lower end of the range 0.9–1.7 as recommended by WHO guidelines and should be sensitive to clinically relevant deficiencies of clotting factors II V VII and X.

Aims: To evaluate a new high sensitivity human recombinant thromboplastin liquid reagent from Diagnostic Grifols (DG PT RecombiLIQ) on Q analyser

Methods: A reference range of 10.2–13.6 was derived from PTs of 20 normals and used to assess sensitivity to factor deficiencies. We studied 23 patients with congenital single deficiency of FV, FVII or FX (below 50 IU dL⁻¹): 30 patients receiving UFH; 30 patients receiving LMWH; 20 patients receiving rivaroxaban.; pooled normal plasma spiked with apixaban or dabigatran. We also studied mixtures of normal/factor deficient plasma, prepared according to CLSI guidelines.

Results: The level of deficiency associated with PT prolongation using DG PT RecombiLIQ using mixtures of deficient and normal plasma was: FII – 45 IU dL⁻¹; FV – 59 IU dL⁻¹; FVII – 62 IU dL⁻¹; FX – 59 IU dL⁻¹. In patients with congenital deficiencies the PT was prolonged with DG-PT RecombiLIQ in 22/23 cases with a PT of 13 s in the presence of 49 IU dL⁻¹ FX in the other. Mean PT ratios were DG-PT RecombiLIQ – 2.16; Inn – 1.72 ($P < 0.0002$). DG-PT RecombiLIQ had a manual ISI of 0.95 and ISI of 0.79–0.80 for 3 lots when used with Q haemostasis analyser. PTs were unaffected by UFH or LMWH up to at least 1.0 IU mL⁻¹ in patient samples. Rivaroxaban concentration of 160–200 ng mL⁻¹ prolonged PT with DG PT RecombiLIQ. PT was prolonged ~2 s by 400 ng mL⁻¹ apixaban and ~5 s by 150 ng mL⁻¹ dabigatran

Conclusion: DG PT RecombiLIQ is a liquid formulation which is therefore convenient to use, complies with WHO guidelines in relation to use for monitoring vitamin K antagonists and is suitable for use in the investigation of subjects with bleeding disorders affecting the PT.

Disclosure of Interest: A. Woolley: None declared, S. Kitchen Speaker Bureau of: Grifols.

PO137-WED

Evaluation of a new, highly sensitive loci D-dimer method for the detection of very low concentrations in human citrated plasma*

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Background: D-dimer is an important marker for the diagnosis of thromboembolic events with clinical potential for assessing cardiovascular thrombotic risk and monitoring anticoagulation therapy. We provide preliminary performance characteristics of a new, highly sensitive immunoassay* for the quantitative determination of D-dimer based on LOCI® Advanced Chemiluminescence technology. **

Aims: Characterization of the assay.

Methods: LOCI technology is a homogeneous, chemiluminescent immunoassay approach that consists of a streptavidin-coated Sensi-bead particle, a Chemibead particle with immobilized antibody, and a second biotinylated antibody for immunometric assays. In the assay, the analyte forms bead-aggregated immune complexes via monoclonal antibodies, thereby generating a signal chain leading to a delayed, analyte-concentration-dependent luminescence emission.

Results: We have characterized the assay regarding stability, reproducibility, linearity, potential interfering factors, and comparability to the Siemens INNOVANCE® D-Dimer assay.

Conclusion: We conclude that the new, highly sensitive D-dimer assay based on LOCI technology provides reliable results regarding precision, linearity, interference, and stability across a wide dynamic assay range using only 5 µL sample volume. *Under development. Not available for sale. Product availability may vary from country to country and is subject to varying regulatory requirements. **LOCI and all associated marks are trademarks of Siemens Healthcare Diagnostics Inc. or its affiliates.

Disclosure of Interest: M. Ehm Employee of: Siemens Healthcare Diagnostics Products GmbH, D. Kraul Employee of: Siemens Healthcare Diagnostics Products GmbH, A. Stangl Employee of: Siemens Healthcare Diagnostics Products GmbH, U. Kuhn Employee of: Siemens Healthcare Diagnostics Products GmbH, O. Pohlack-Marsch Employee of: Siemens Healthcare Diagnostics Products GmbH, P. Vesper Employee of: Siemens Healthcare Diagnostics Products GmbH, M. Preis Employee of: Siemens Healthcare Diagnostics Products GmbH, E. Boucsein-Mueller Employee of: Siemens Healthcare Diagnostics Products GmbH, U. Krupka Employee of: Siemens Healthcare Diagnostics Products GmbH

PO138-WED

Silica clotting time (SCT) vs. dilute venom Russell viper (DVRV) and activated partial thromboplastin time (APTT) in the diagnosis of antiphospholipid antibodies syndrome

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Background: Due the heterogeneity of antibodies with lupus anticoagulant (LA) effect, the diagnosis of the APS is a challenge for any hemostasis laboratory.

Aims: The study aim was to contrast our routine test for LA detection: dVRV, and aPTT-SP vs. SCT in patients APS suspicious in 4 groups: systemic lupus erythematosus (SLE), APS suspected, any other autoimmune diseases (AID), and patients with/without clinical rheumatological symptoms without clinical diagnosis (PWCD).

Methods: We collected and studied consecutive plasmas from 95 patients (79 women 16 men, age 14–67 years old), for which a LA detection was requested. We performed it via our AL routine test; the results were contrasted against SCT (providing by IL Werfen Mexico). All the tests were run on an ACL 9000 automated coagulometer (IL Werfen). Median ± SD and 99 percentile for LA Screen/LA Confirm normalized ratio: 1.23 ± 0.11, 1.48; aPTT-SP ratio: 1.0 ± 0.12, 1.25.

Results: Normal SCT values were established in 40 healthy subjects (13 women), same parameters for normalized SCT ratio: 1.0 ± 0.11, 1.21. Values above the 99 percentile were considered as positive (AL+). APS group presented normalized ratio significantly higher than the one in the AID group. From 95 patients, 3/7 from APS group, were previously classified LA+ vs. 5 by SCT; 5/29 from SLE group, were classified LA+ vs. 9 by SCT; 4/24 from AID group, were classified LA+ vs. 8 by SCT; 4/35 from PWCD group were classified LA+ vs. 5 by SCT. Insofar as the SCT test performance, 14/16 (AL+ by routine LA test) were AL+ by SCT and 66/79 (AL- by routine) were AL- by SCT; thus SCT sensitivity was 87.5%, and SCT specificity was 86.5%.

Conclusion: The positive result in the LA test implies an elevated risk of thrombosis, those to determine its presence is outstanding for clinical judgment treatment. In this study SCT showed a greater capacity to detect LA than our routine test, however, we cannot forget dVRV, being that there were 2 patients that just had AL+ by this assay.

Disclosure of Interest: None declared.

PO139-WED

Discordance between activated partial thromboplastin time and heparin level measured with anti-Xa assay in antithrombin-deficient plasmaYi AK¹, Stevic I², Gantioqui J³, Lau KK⁴, Roberts R⁵, Chan AK⁶ and Chan HH⁷¹Health Sciences, McMaster University, Hamilton; ²Clinical Biochemistry and Genetics, Diagnostic Services of Manitoba, University of Manitoba, Winnipeg; ³TAARI, McMaster University, Hamilton, Canada; ⁴Pediatrics, Hong Kong University - Shenzhen Hospital, Shenzhen, China; ⁵Clinical Epidemiology and Biostatistics; ⁶Pediatrics; ⁷Medicine, McMaster University, Hamilton, Canada**Background:** Patients with antithrombin (AT) deficiency are predisposed to thromboembolism. If these patients are treated with unfractionated heparin (UFH), it will be problematic because the therapeutic effects of UFH are AT-dependent.**Aims:** To evaluate aPTT and anti-Xa assays for the measurement of UFH in AT-deficient plasma**Methods:** The aPTT assay was performed in AT-deficient plasma prepared by mixing platelet-poor plasma with plasma totally depleted of AT to attain samples with predefined AT concentration (AT%). These samples were spiked with UFH at 0.3, 0.4 or 0.5 units mL⁻¹, followed by pre-incubation with aPTT-SP, then activated with 10 mM Ca²⁺ at 37 °C. Clotting time (CT, time to reach half-maximum turbidity) was monitored at 350 nm. A chromogenic anti-Xa assay was performed in similarly prepared AT-deficient plasma samples spiked with UFH described above. The samples were diluted 1:20 in Tris buffer and then incubated with 40.6 nM FXa at 37 °C for 4 min. Using S2222 substrate, the residual Xa activity was measured at 405 nm in a spectrometer. The equivalent UFH concentrations were extrapolated from a standard curve using the same UFH preparation. A modified *t*-test was used to determine the first dose level of AT% being significantly different as compared with the baseline with 100% AT.**Results:** With aPTT assay, CT for a specific UFH concentration plateaued at higher AT% but then significantly dropped when AT was reduced < 50%. In contrast, for each UFH concentration, chromogenic anti-Xa assay demonstrated a linear decrease in UFH activity proportional to AT%.**Conclusion:** Monitoring UFH therapy using aPTT is adequate when AT% in plasma is > 50%. However, measuring the anti-Xa activity of UFH is dependent on AT%. The AT% may reduce in patients not only with inherited AT deficiency, but also with acquired diseases. Correction by the addition of a fixed amount of extrinsic AT to the testing samples will not suffice because the amounts of added AT will depend on the degree of deficiency in the original samples.**Disclosure of Interest:** None declared.

PO140-WED

Multicenter study of two new coagulation analyzers in comparison to a predicate device already in the US-Market, using Siemens Healthcare ReagentsWeik MR¹, Birschmann I², Eby CS³, Bettina K-M⁴, Manzella SM⁵, Peerschke EIB⁶ and Pipe SW⁷¹HC DX HHS QM CA HSRE, Siemens Healthcare Diagnostics Products GmbH, Marburg; ²Institut für Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum Nordrhein-Westfalen Universitätsklinik der Ruhr-Universität Bochum, Bad Oeynhausen, Germany; ³Department of Pathology & Immunology, Washington University School of Medicine, Saint Louis, MO, USA; ⁴Haemostasis Center, University Hospitals Gießen and Marburg, Gießen, Germany; ⁵Clinical Chemistry and Immunochemistry, Point-of-Care testing and Support Services, WellSpan York Hospital, York, PA; ⁶Department of Laboratory Medicine, Memorial Sloan Kettering Cancer Center (MSKCC) and Weill Cornell Medical School, New York, NY; ⁷Division of Pediatric Hematology and Oncology, University of Michigan, Ann Arbor, MI, USA**Background:** We compared two new automated coagulation analysers, Sysmex CS-2100i and Sysmex CS-5100 Systems* (Siemens Healthcare Diagnostics, CS-2100i and CS-5100) with Siemens reagents, vs. the Sysmex CA-1500 System (Siemens Healthcare Diagnostics, CA-1500).**Aims:** Performance characteristics of CS-2100i and CS-5100 to CA-1500, for Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Fibrinogen-, Antithrombin- (AT) and D-dimer were compared.**Methods:** 3 US laboratories participated in the method comparison (MC) studies. Result-comparability was investigated using leftover samples(**). MC of CS-5100 and CS-2100i vs. CA-1500 were based on a total of 2601 and 2515 results respectively. Precision was performed at 2 German and 1 US site and included 47 samples covering the total clinical reportable range. The complete data set contained 28,000 results. In addition, performance data for reference intervals, Factor- and Lupus-sensitivity and MC of results when testing fresh vs. frozen samples and when using different pipetting modes were examined.**Results:** Analysis of MC-data by Passing-Bablok regression (PBR) and Difference plot revealed very good agreement to CA-1500, showing PBR slopes between 0.971 and 1.077 and correlation coefficients ≥ 0.994 . CV for BETWEEN SITE precision varied from 0.00% to 5.16%, with a median CV of 0.88%.**Conclusion:** Results of both systems were in very good agreement with CA-1500. Precision for the new devices/reagents combination showed very low CV values. Combined with the improved functionality, the CS-2100i and CS-5100 provide high performance, quality and efficiency to the mid- to high-volume coagulation laboratories.

(*) The products/features (mentioned herein) are not commercially available in all countries. Due to regulatory reasons their future availability cannot be guaranteed. Please contact your local Siemens organization for further details.

(**) Donors gave informed consent and review boards were involved.

Disclosure of Interest: M. Weik Employee of: Siemens Healthcare Diagnostics Products GmbH, I. Birschmann: None Declared, C. Eby: None Declared, K.-M. Bettina: None Declared, S. Manzella: None Declared, E. Peerschke: None Declared, S. Pipe: None Declared.

PO141-WED

Evaluation of the new qsmart from grifols in comparison to the qhemostasis analyzer

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Background: The QSmart is a new automated coagulometer from Grifols, able to test haemostasis parameters using coagulometric, chromogenic and immunoturbidimetric assays, and suitable for low to medium volume labs.

Aims: To assess the QSmart for routine laboratory use and in comparison to the Grifols QHemostasis Analyzer

Methods: We determined PT/INRs with Q and QSmart using DG-PT or DG-PT L REC reagents in normal subjects ($n = 30$) and warfarinised patients ($n = 79$). APTTs with DG-APTT Synth were determined with the QSmart in 30 normal subjects, and 35 subjects with a single clotting factor deficiency of FVIII/IX/XI or XII. Factor VIII Factor IX and Factor V assays were performed (QSmart) on 21 normal subjects using three test sample dilutions prepared and assessed for parallelism automatically.

Results: The normal ranges for PT and APTT were practically identical for Q and QSmart. The mean INRs with QSmart and Q were as follows: DG-PT – 2.77 and 2.85 (not significant, ns); DG-PT L Rec – 3.18 and 3.19 (ns). All subjects with FVIII, FIX or FXI below 30 IU dL⁻¹ had prolonged APTTs with QSmart and DG-APTT Synth. The mean regression correlation (r) of normal sample plots (with CV of the three individual test dilutions in brackets) were as follows: FVIII – 0.998 (7.1%); F IX – 0.999 (4.7%); FV – 0.997 (7.7%). All r values were > 0.99 . Sample and reagent loading was simple, software was user friendly (meaning that operator switching between Q and QSmart was straightforward), and data handling was clear and responsive

Conclusion: The QSmart analyser with DG reagents gave results which were interchangeable with those on the Q Hemostasis Analyzer. The PT and APTT were suitable to screen for the presence of bleeding disorders. Automatic plots of three dilution factor assays showed good linearity and parallelism. We conclude that the QSmart in combination with the reagents evaluated is suitable for routine use alongside QHemostasis Analyser.

Disclosure of Interest: A. Woolley Speaker Bureau of: Grifols, S. Kitchen: None Declared.

PO142-WED

Hemocompatibility of estetrol and estradiol coupled to cyclodextrinesLaloy J^{1,2}, Palazzo C³, Alan L^{1,2}, Foidart J-M⁴, Piel G³ and Dogné J-M^{1,2}*¹Department of Pharmacy, University of Namur; ²Namur Nanosafety Centre, Namur; ³Laboratoire de Technologie, University of Liège; ⁴University of Namur, Liège, Belgium*

Background: In order to prevent damages to premature babies, we want to develop liposomes based estetrol and estradiol to increase their concentration into the brain. These liposomes will be administered *in vivo* by iv injection. The study of their hemocompatibility if of major importance. In fact, a dysfunction of the hemostasis can lead to slow down or even to completely stop the circulation of the blood. It is therefore primordial to study the hemocompatibility of nanoparticles (NPs).

Aims: The aim of this study is the evaluation of the hemocompatibility of estetrol and estradiol-based liposomes on hemolysis, platelet function and coagulation.

Methods: Crysmeb[®] and hydroxypropyl- β -cyclodextrin (substitution degrees of 0.63) HEPES buffer solutions were prepared at different

concentrations. An excess of estradiol or estetrol was added to the cyclodextrin solutions and the dispersion was stirred at 140 rpm at 25°C for 24 h. Then the unsolubilized hormone was separated by filtration.

Various techniques assessing erythrocytes integrity, activation and aggregation of the platelets or the impact of NPs on coagulation cascade were investigated on human blood.

Results: Estetrol and estradiol alone or coupled with cyclodextrines induced no hemolysis. Estetrol, with or without cyclodextrins had no impact on platelet function and coagulation. Complexes cyclodextrin-estradiol avoid platelet aggregation and extend the blood coagulation.

Conclusion: Estetrol and complexes cyclodextrin-estetrol are hemocompatible. Complexes cyclodextrin-estradiol show anti-aggregating and anti-coagulant properties.

Disclosure of Interest: None Declared.

PO143-WED

Evaluation of prolonged PT/APTT tests in presurgical plasma samples

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Background: Prothrombin time (PT) and activated partial thromboplastin time (APTT) are the essential tests used for screening clotting factor deficiencies and lupus anticoagulant as well as monitoring anticoagulation drugs in routine clinical practice. However, mild prolongations of PT or APTT may not always indicate a clotting factor deficiencies.

Aims: In this study, we examined the relation of prolonged PT and APTT tests with factor levels in routine blood samples.

Methods: Clotting factor levels were measured in 90 sequential plasma samples with prolonged PT or APTT and normal lupus anticoagulant tests by ACL TOP 700 coagulation analyser (IL, USA) in our laboratory. Factor deficient plasma samples were stratified into four groups according to the factor (F) levels; group 1 ($F < 0.05$ IU dL⁻¹, $n:24$), group 2 ($F = 0.05-0.30$ IU dL⁻¹, $n:16$), group 3 ($F = 0.30-0.50$ IU dL⁻¹, $n:20$) and group 4 ($F > 0.50$ IU dL⁻¹, $n:30$).

Results: Of the 90 samples with prolonged PT or APTT, sixty (66%) decreased factor levels (< 0.50 IU dL⁻¹) while the levels were above 0.50 IU dL⁻¹ in 30 (34%) samples. The median APTT value was significantly higher in the group 1, group 2 and 3 vs. group 4 (49 s [26–74 s] vs. (33 s [24–55 s]) vs. (30 s [22–46 s]) vs. (24 s [20–30 s]) ($P:0.001$) while the median PT value was not significantly different among all groups ($P:0.64$). The peak APTT value in group 4 was 30 s, which was 4 s longer than the upper limit of our laboratory's normal range. In 30 samples (34% of all samples) PT or APTT was mildly prolonged and factor levels were normal. All of these samples belonged to patients undergoing presurgical screening.

Conclusion: Our data suggest that measurement of clotting factor levels may not be necessary in samples with mildly (> 4 s) prolonged APTT value, particularly in cases of presurgical screening.

Disclosure of Interest: None Declared.

PO144-WED

Validation of new automated latex-immunoturbidimetric assay for measuring VWF activityRimac V¹, Vojak SS², Herak DC¹ and Zadro R^{1,3}¹Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb; ²Department of Laboratory Diagnostics, County General Hospital Pozega, Pozega; ³Faculty of Pharmacy and Biochemistry Zagreb, Zagreb, Croatia

Background: Ristocetin cofactor activity of von Willebrand factor (VWF:RCO) and VWF antigen concentration are still essential laboratory assays for the diagnosis of von Willebrand's disease (VWD). As VWF:RCO has poor reproducibility and sensitivity to low levels of VWF, new generation assays for measurement of VWF activity (VWF:Ac) are developed. They are based on binding VWF to recombinant GPIb which in turn binds to particle-fixed anti-GPIb antibodies, and do not contain ristocetin.

Aims: The aim was to validate the new VWF:Ac assay and compare obtained results with VWF:RCO assay.

Methods: We have validated the performance of a new automated latex-immunoturbidimetric assay (INNOVANCE VWF Ac) on Sysmex CS2000i and on BCS analyzer (Siemens Healthcare Diagnostics, Germany). Within-run and between-run imprecision were estimated in normal and pathological range using Control plasma N (CPN) and Control plasma P (CPP). Low-range calibration curve was tested using CPP prediluted 1/3 on Sysmex CS2000i. Method comparison was performed in normal and clinical samples with VWF:RCO (BC von Willebrand Reagent) using Passing and Bablok regression.

Results: Low coefficients of variations (CVs) for within-run and between-run imprecision were obtained on both analyzers using CPN (BCS within-run CV 1.5%, between-run CV 3.0%; CS2000i within-run CV 2.0%, between-run CV 3.8%) and CPP (BCS within-run CV 2.2%, between-run CV 4.4%; CS2000i within-run CV 3.7%, between-run CV 4.4%). Even for CPP 1/3, within-run and between-run imprecision CVs were 3.6% and 3.5%, respectively. Normal and clinical samples demonstrated good correlation between VWF:RCO and VWF:Ac on both analyzers (BCS $y = 0.88x + 5.77$, $r = 0.968$, $P < 0.001$; CS2000i $y = 1.05x - 0.04$, $r = 0.987$, $P < 0.001$).

Conclusion: Considering low CV values, VWF:Ac assay had satisfactory accuracy and precision on both analyzers (even for low VWF activities) and good correlation with VWF:RCO. The performance of VWF:Ac assay makes it suitable for the laboratory diagnosis of VWD.

Disclosure of Interest: None Declared.

PO145-WED

Laboratory evaluation of patients with undiagnosed bleeding disorders

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Background: The evaluation of patients with a bleeding tendency represents a challenge as the routinely available tests for evaluating bleeding disorders are limited; complicating the laboratory determination of the clinically observed bleeding tendency. As a result, some bleeding disorders remain undiagnosed.

Aims: To evaluate whether global coagulation tests would contribute to the laboratory analysis of patients with undiagnosed bleeding disorders.

Methods: Patients were evaluated for coagulation and fibrinolysis activities by thrombin generation test (TGT) and euglobulin lysis time (ELT). In addition, plasma activity of factor (F) XIII, plasminogen, a-2 antiplasmin (a2AP), plasminogen activator inhibitor-1

(PAI-1), and thrombin activatable fibrinolysis inhibitor (TAFI) were also obtained.

Results: Forty-five patients were included. Eight percent presented a mild bleeding disorder and 20% a moderate bleeding disorder. The TGT results were similar between patients and controls. ELT results however, were lower in patients than in controls, both before (median = 175 vs. 250 min, respectively, $P = 0.003$) and after arm constriction (median = 145 vs. 115 min, respectively, $P \leq 0.001$), suggesting that they were experiencing hyperfibrinolysis. Interestingly, patient's median TAFI activity was higher than in controls (21.2 vs. 19.46 $\mu\text{g mL}^{-1}$, $P = 0.016$). However, plasminogen, a-2AP, PAI-1, and FXIII activities did not differ between the groups.

Conclusion: Global coagulation and fibrinolysis tests proved to be limited in detecting the disorders of hemostasis in some patients with a relevant bleeding tendency and may not be adequate to address their bleeding risk. Bleeding scores are currently the available medical approach for the evaluation of these patients.

Disclosure of Interest: None Declared.

PO146-WED

Comparison of methods of measurement of factor VIII inhibitors in a new haemophilia care centerArias M¹, Sueldo E¹, Avalos M¹, Do Nascimento P², Guerrero G² and Baques A²¹Hematology Laboratory; ²Hemophilia, Unidad Asistencial Por Mas Salud Dr. Cesar Milstein, Ciudad Autonoma de Buenos Aires, Argentina

Background: Patients with Haemophilia A (HA) under replacement therapy with factor VIII (FVIII) concentrates could develop alloantibodies against FVIII (inhibitors) with progressive specific activity. Bethesda Titration (BT) method has been described many years ago. Nijmegen modification (NMT) has been more recently recommended.

Aims: To compare NMT to BT methods in samples from patients with HA in a new center of Haemophilia.

Methods: 83 patients samples with HA corresponding to 55 patients (49 severe HA, 2 moderate HA, 4 mild HA). Protocol Clinical Laboratory Standards Institute (CLSI) EP 15-A2 was used for comparing methods. BT: Samples were diluted with Owren Buffer and incubated 2 h at 37 °C with normal pooled plasma. Results were expressed as UB/mL. NMT: Samples were diluted with FVIII deficient plasma preheated at 58 °C for 1.5 h. Then the mixture was incubated with plasma Calibrator (Siemens) at 37 °C for 2 h. Results were expressed as UBN/mL. The cut off to consider presence of inhibitor was 0.6 UB-UBN mL⁻¹. FVIII assay was performed by one-step coagulometric assay with reagents from Siemens. Autoanalyzer: STA COMPACT STAGO Statistical analysis InfoStat /L.

Results: 83 samples show a Pearson correlation coefficient (r) of 0.99, k (concordance) of 0.95, the means were BT: 24.37 $\pm$ 3.99 vs NMT: 32.16 $\pm$ 4.60 (paired T test comparison, $P = 0.5241$). Seventy-six samples with titles < 0.6 showed a r of 0.67 k 0.56, the means were BT: 0.32 $\pm$ 0.30 vs NMT: 0.20 $\pm$ 0.21, $P < 0.0001$, but seven samples with titers > 0.6 showed a r of 0.99 k 0.94, the means were BT: 43.79 $\pm$ 78.09 NMT: 52.47 $\pm$ 105.92 (paired T test comparison, P 0.4847).

Conclusion: Results of anti Factor VIII inhibitor titers obtained by using the two methods evaluated were statistically comparable. Moreover, samples in which the inhibitor is detected the correlation was excellent. Titles discrepancies and low concordance were observed in samples negative for inhibitor.

Disclosure of Interest: None Declared.

PO147-WED

The normal range of the thrombodynamics values for apparently healthy pregnant women

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Background: A number of adaptive processes including rapid changes of hemostasis occur in women during pregnancy, resulting in the hypercoagulant state.

Aims: To determine the range of the Thrombodynamics (TD) assay values in pregnancy and to compare them with the normal reference range.

Methods: The TD assay (with its parameters of lag time (Tlag), initial (Vi) and stationary (Vst) clot growth velocity, clot size and clot density) and routine coagulation tests (aPTT, PT, TT, AT, INR, Fg, D-dimer, SFMC) were carried out in plasma samples from apparently healthy pregnant women: 84, 113 and 94 women during the 1st, 2nd and 3rd trimesters, respectively (median age 30 years for all groups), without pathologies, burdened family history, or treatment. Exclusion criteria were a complication of pregnancy connected to the hemostatic pathology, life-threatening miscarriage, endocrine disorder, or intake of the hemostasis affecting drugs.

Results: We have found a gradual increase of the TD parameters with the duration of gestation indicating the hypercoagulant state (see Table).

Trimester:	1st	2nd	3rd	Normal range
Vi (µm/min)	[41.6–61.1]	[42.8–58.7]	[42.3–58.7]	[38.0–56.0]
Vst (µm/min)	[22.8–36.2]	[21.4–37.0]	[22.5–35.3]	[20.0–29.0]
Clot size (µm)	[985–1290]	[908–1323]	[908–1345]	[800–1200]
Clot density (a.u.)	[17758–32295]	[19329–32329]	[20894–35024]	[15000–32000]
Tlag (min)	[0.75–1.55]	[0.66–1.20]	[0.65–1.50]	[0.60–1.50]

The aPTT, PT, TT, AT, and INR values corresponded to the normal reference range. Fibrinogen, SFMC, and D-dimer were slightly increasing during pregnancy.

Conclusion: Most parameters of the TD assay in pregnant women approached the values typical for the hypercoagulant state.

Disclosure of Interest: None Declared.

PO148-WED

Factor VIII inhibitor testing, interpretation and standardization – it's complicated

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Background: The foundation for inhibitor testing was laid in the early 1970s using the most prevalent FVIII inhibitors. At this time, neither the biological nature of FVIII nor the mechanisms of FVIII inhibition by antibodies were known. Assays were designed assuming a bimolecular reaction in which FVIII is destroyed. Inhibitors against other coagulation factors are tested in a similar approach.

Aims: The relationship between inhibitor properties and assay results must be fully understood to allow valid interpretation or clinical decisions. This was not achieved yet.

Methods: The influence of assay variables on inhibitor testing was tested and interpreted on the base of the more appropriate receptor-ligand model. This includes antigen concentration, antibody dilution, incubation time and the affinity of the interaction. In the case of FVIII inhibitors the influence of VWF was investigated.

Results: For non-FVIII inhibitors the Bethesda/Nijmegen assay is a valid approach to quantify inhibitor potency. The affinity of the antibody-antigen interaction plays an insignificant role. It is even possible to simplify the calculation of inhibitor potency.

The uniqueness of FVIII inhibitors, besides its interaction with VWF, lies in the low concentration of FVIII matching the dissociation constant of a typical antibody. Here, the antibody-antigen affinity plays a major role as low-affinity antibodies show a significant different behavior as medium- or high-affinity antibodies.

Conclusion: While inhibitor testing for non-FVIII antibodies bases on a straightforward relationship between inhibitor potency and antibody concentration, this does not hold true for FVIII inhibitors. Interpretation of anti-FVIII inhibitor results must be seen as an educated guess. Due to the significant influence of assay-related variables on FVIII inhibitor testing, standardization requires very strict conditions.

Disclosure of Interest: None Declared.

PO149-WED

Can the overall haemostatic potential assay predict bleeding and thrombosis risk in warfarinised patients?

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Background: Patients on warfarin not uncommonly require reversal for surgical procedures. An important threshold for safe intervention is INR ≤ 1.5. However, whether the INR is a good predictor of surgical thrombohaemorrhagic risk is somewhat unchallenged. Global assays such as overall haemostatic potential (OHP) may be a better predictor.

Aims: To assess OHP in patients on warfarin, pre- and post reversal with intravenous vitamin K (vitK-IV). Correlate OHP with INR, vitK-dependent factor levels and post-surgical thrombohaemorrhagic outcomes.

Methods: Platelet poor plasma derived from citrated blood from patients on stable long-term warfarin, immediately pre- and 18-24 h post-reversal with vitK-IV 3 mg, was assessed for OHP. Low and high levels were based on quartiles of 34 healthy controls. Results were correlated with INR, FII, VII, IX, X and fibrinogen.

Results: In this correlative substudy, 56 patients underwent 62 reversals. The most common OAC indications were atrial fibrillation (65%)/previous TE (23%). Median (range) INR on warfarin was 2.3 (1.1-3.3) and post-vitK-IV 1.2 (1-1.8). OHP increased following reversal (e.g. median OCP 14.5 before vs 51.0 after reversal, $P = 0.005$) but did not normalise. 87% of warfarinised samples were in the bottom quartile for OHP and 61% after reversal. OHP levels and quartiles correlated with INR/factors on warfarin and did not correlate with absolute/percentage change in INR or factors after reversal. OCP on warfarin was lower in a subset of patients ($n = 3$) with peri-operative bleeding.

Conclusion: Patients on long-term warfarin showed suppressed fibrin generation, which increased after vitK-IV reversal but remained low compared with normal controls, a finding not explained by changes in INR or vitK-dependent factors. The OHP assay is dependent on other components of haemostasis, including fibrinogen and factor VIII, which may explain this finding. A larger prospective study would be

required to determine whether the OHP can predict thrombohaemorrhagic risk in warfarinised patients.

Disclosure of Interest: None Declared.

PO150-WED

Detection of hypercoagulation in hip arthroplasty patients

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Background: Deep venous thrombosis (DVT) is a common complication after surgery and anticoagulant prophylaxis can't eliminate it at all.

Aims: To investigate coagulation tests potency to detect post-op hypercoagulation, following that effect of anticoagulant and condition after discontinuation of a drug.

Methods: 41 hip arthroplasty patients (pts) were enrolled. Prophylaxis performed by low molecular weight heparin (LMWH) enoxaparin 40 mg, with 1st injection 12 h before a surgery, then once per day for 10 days starting at 12 h after a surgery. Blood was collected before surgery and 1st LMWH, after surgery before 2nd LMWH, on 10th day before the last LMWH shot, and on 30th post-op day. Thromboelastometry (TEM), thrombodynamics (TDx), activated partial thromboplastin time (APTT), prothrombin by Quick (Quick), D-dimer (norm <250 ng mL⁻¹), thrombin-anti-thrombin complex (TAT) were done.

Results: Trauma hypercoagulation after surgery were detected by TDx: spatial clot growth rates (V) significantly increased from 29 to 41 um min⁻¹ (norm 20-30); the prevalence of spontaneous clotting increased from 10% to 73% of cases ($P < 0.05$). APTT, Quick, TEM detected shifts towards to the range of hypocoagulation. TAT was not changed. D-dimer increased from 145 to 800 ng mL⁻¹.

Effect of LMWH on the 10th post-op day - TDx, TEM, APTT and Quick data demonstrated restoration to the baseline level or normal range. TAT was not changed. D-dimer level decreased to 450 ng mL⁻¹.

On 30th day APTT, Quick and TEM have no changes compared to 10th post-op day. D-dimer got back to a normal range. TDx revealed increased V from 24 to 33 um min⁻¹, and prevalence of spontaneous clotting changed from 9% to 39% cases ($P < 0.05$). TAT were increased by that time also from 9% to 39%.

Conclusion: TDx is able to detect hypercoagulation in post-op period and after termination of anticoagulation. We suppose that TDx potentially might serve as an instrument for recognizing the group of pts at risk of DVT.

Disclosure of Interest: L. Parunov Employee of: Hemacore LLC, I. Taranenko: None Declared, A. Momot: None Declared, F. Ataullakhanov Employee of: Hemacore LLC, I. Serebriyskiy Employee of: Hemacore LLC.

PO151-WED

Heparin and protamine confound factor activity and inhibitor testing while on cardiopulmonary bypass

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Background: Cardiovascular surgery requiring cardiopulmonary bypass is becoming more common in the ageing population with

hemophilia. We describe investigations triggered by bleeding in a patient with hemophilia undergoing cardiac surgery necessitating intra-operative factor activity and inhibitor testing. The effect of heparin and protamine on factor VIII (FVIII), IX (FIX) activities and their inhibitor assays are not well understood.

Aims: To reveal the in vitro effect of heparin, protamine and protamine:heparin combinations on FVIII/FIX activities and their Bethesda inhibitor assays.

Methods: In vitro spiking experiments were conducted with heparin, protamine or protamine:heparin combinations mimicking expected *in vivo* ratios. Assays that were performed include FVIII/FIX activities and their Bethesda inhibitor assays.

Results: Heparin alone in a linear dose-dependent fashion prolonged the one-stage aPTT-based FVIII and FIX assays, with FIX being more sensitive to heparin than FVIII. Peak FVIII inhibitor titer at 4 BU mL⁻¹ and FIX inhibitor titer at 9 BU mL⁻¹ were seen within the range of heparin concentrations tested. Unexpectedly, protamine had a biphasic effect on FVIII and FIX activity with an inflection point at 100 µg but did not cause detectable inhibitor titers in the range tested. False positive factor inhibitor titers were not observed at protamine:heparin ratios of 0.2:1 to 0.4:1. At higher ratios, false-positive inhibitors begin to appear, also with a biphasic pattern.

Conclusion: These preliminary findings suggest caution in the interpretation of factor activity and inhibitor results when heparin and/or protamine may be present in the sample. Our data imply that the protamine:heparin interaction influences aPTT-based assays with complex stoichiometry. While we cannot entirely exclude artifact due to in vitro spiking, our data thus far suggest that intra-operative factor and inhibitor aPTT-based assays in the presence of heparin and/or protamine may have unpredictable effects.

Disclosure of Interest: None Declared.

PO152-WED

Hemostasis alterations associated to complications in sickle cell syndrome

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Background: Sickle cell syndrome (SCS) is one of the most common inherited disorders worldwide, includes a group of hemolytic anemia associated to the presence of hemoglobin S, which is characterized by acute pain episodes and progressive damage of different organs. In Venezuela SCS frequency ranges between 0% and 7%. Some patients with sickle cell syndrome have shown an increased risk of stroke, pulmonary hypertension, avascular necrosis of joints, acute chest syndrome and pregnancy complications. Associated with abnormalities of Hemostasis such as a hypercoagulable state.

Aims: Evaluated biomarkers of coagulation and fibrinolysis as risk factors for thrombotic manifestations.

Methods: the present study evaluated the changes in Thrombin Generation (GT) method THROGA (Jena, Germany), concentration of Von Willebrand Factor (vWF); and The coagule lysis evaluation was performed by determining the time of lysis (TL), the rate of coagule lysis (RL) and rate of polymerization (RP).

Results: 52 patients Venezuelans with sickle cell syndrome classified as mild, moderate and severe disease and 30 healthy controls All signed a valid consent were evaluated. Significant differences ($P < 0.05$) was observed in the levels of vWF, GT, TL, RL and RP between patients with sickle cell syndrome (Hb SS) and healthy controls. Positive correlation between vWF and GT with severe disease phenotype was observed (vWF $r = 0.575$, $P = 0.0011$; GT $r = 0.6836$ $P = 0.0005$).

Conclusion: This study supports the association of coagulation activation and hypercoagulable state with thrombotic manifestations and

severe disease phenotype, these parameters should be considered as risk factors for thrombotic manifestations. More research is needed to evaluate the contribution of coagulation and fibrinolytic activity to clinical complications in SCS.

Disclosure of Interest: None Declared.

PO153-WED

Activated partial thromboplastin time (APTT) clot waveform analysis (CWA) on SYSMEX CS 2000 helps differentiate milder from severe phenotype in patients with severe hemophilia A

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Background: Since its first report on the assessment of global haemostasis in severe hemophilia in 2002, reports of the use of CWA have been infrequent because coagulometers offering this option were limited. We have assessed CWA on the CS2000(Sysmex,Kobe,Japan) a coagulometer which detects decrease in transmittance at 660 nm during the fibrin clot formation and this rate or Velocity is Minimum of 1st derivative (min1) and the acceleration as Minimum of 2nd derivative (min2).

Aims: To assess the ability of CWA on CS2000to detect small changes in factor activity of spiked FVIII deficient plasma and evaluate its usefulness in differentiating the bleeding heterogeneity in patients with severe hemophilia A (SHA) with FVIII:C less than 0.01 IU mL⁻¹.

Methods: CWA reference range was established using 133 healthy blood donors. CWA of APTT clot curves of plasma obtained from 45 patients with SHA who were part of an ongoing study and had not received treatment with FVIII products for at least 1 week before the time of sampling. Annual bleeding rate, number of joints involved, HJHS and Petterson score were used to categorize the mild phenotype among them.

Results: CS2000 CWA reference range for APTT was: (min1/min2 = 1.40 - 3.40/ 0.20 - 0.60). Evaluation of FVIII deficient plasma spiked with rFVIII also showed good linearity of min1 and min2 extending to levels of 0.001iu/mL even though the lowest limit of detection of FVIII:C on CS2000 was only 0.003 IU mL⁻¹. Six of the 45 patients with SHA were categorized as having a mild phenotype. Both min1 and min2 values (mean - 0.95; Range 0.53-1.44/ mean - 0.10; Range 0.06 - 0.19) obtained on these patients were higher than the patients with severe phenotype (mean - 0.56; Range 0.33 - 1.26/ mean - 0.05; Range 0.02 - 0.14; $P = 0.002$).

Conclusion: The APTT CWA on the CS2000 is sensitive to a wide range of FVIII activity and could differentiate milder from severe phenotype in patients with severe hemophilia A. This is the first time an APTT CWA study was done on CS2000.

Disclosure of Interest: None Declared.

PO154-WED

Blood coagulation tests: review of validation requirements for FDA clearance

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Background: Although immediate clot formation is a critical feature of the hemostatic system, excessive clot formation outside of wound healing poses serious medical consequences due to the obstruction of blood flow. The increasing number of blood coagulation disorders

and resulting high demand for blood testing prompts many test developers to create and market new coagulation instruments and assays.

Aims: This presentation will examine FDA performance requirements and the resulting analytical and clinical testing necessary to demonstrate safety and effectiveness of coagulation devices before obtaining FDA clearance and introduction to the US market.

Methods: The importance of identification of an appropriate intended use such as defining patient population, sample matrix and clinical settings will be discussed. Required performance testing and comparative studies with the predicate device will be outlined. Factors affecting device performance such as test modalities, environmental conditions, maintenance requirements to include cleaning and disinfection will be reviewed using an exemplary coagulation device. Additional requirements for reagent stability, assay specificity and sensitivity as well as interference with certain medical conditions and drugs will also be reviewed.

Results: An overview of validation testing requirements and their scientific basis will be provided to for a better understanding of the US regulatory process.

Conclusion: Better understanding of a review process may help tests developers design studies that adequately address FDA requirements.

Disclosure of Interest: None Declared.

Cancer and thrombosis / hemostasis III

PO155-WED

sP-Selectin as a marker of platelet activation in Africans with prostate cancer

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Background: Cancer associated thrombosis is a major cause of morbidity and mortality in cancer patients. sP-Selectin is a cellular adhesion molecule that promotes venous thromboembolism. High plasma levels independently predict VTE in cancer patients and are known to play a major role in tumor metastasis. No study has determined the levels of sP-selectin in prostate cancer patients in Nigeria. Determination of patients at risk will enable prophylactic measures to be instituted.

Aims: To assess the risk of VTE in prostate cancer patients using sP-selectin as a biomarker.

Methods: We prospectively enrolled 88 histologically diagnosed prostate cancer patients and 40 healthy male controls. Full blood count using EDTA samples was done and citrated samples were used for sP-selectin determination using ELISA.

Results: We found significantly reduced haemoglobin in cases than controls (10.79 ± 2.20 vs 13.59 ± 2.20 g dL⁻¹; $P = 0.001$), sP-selectin was significantly higher in cases (57.86 ± 69.92 vs 25.83 ± 9.72 ng L⁻¹; $P = 0.01$) and platelet count showed no significant difference (243.14 ± 150.85 vs 226.80 ± 53.83 ; $P = 0.503$). Twenty four patients had sP-selectin levels >53.1 ng mL⁻¹ (mean 114.3 ± 171.1 ng mL⁻¹). sP-selectin was significantly higher in patients whose BMI was > 25 g m⁻² ($P = 0.03$), those with two or more co-morbidities ($P = 0.02$) and more so in patients with diabetes mellitus ($P = 0.009$). sP-selectin was increased in metastatic cancer patients, although not significantly. No significant correlation was found between sP-selectin level and platelet count ($r = -0.03$).

Conclusion: The findings from this study indicates that sP-selectin is raised in prostate cancer patients. Prostate cancer is associated with a significant risk of VTE. It was not related to the stage of the disease, but rather to increased BMI and presence of co-morbidity. Thrombo-

prophylaxis should be considered in the Cap Patients at risk. The ability of sP-selectin to predict metastasis in prostate cancer needs to be elucidated by further studies.

Disclosure of Interest: None Declared.

PO156-WED

Fractal dimension (DF) as a marker of hypercoagulability, clot structure and 12 month survival in lung cancer

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Background: Venous thromboembolism is common in lung cancer patients, contributing significantly to morbidity, and is the second commonest cause of death. Clinicians are increasingly focused on prevention of cancer-associated thrombosis (CAT). A validated scoring system for predicting CAT exists, but has insufficient predictive value to be used routinely. Fractal dimension (d_f) is a new biomarker used to quantify hypercoagulability and incipient clot structure, potentially identifying those at increased CAT risk.

Aims: d_f has previously been shown to quantify clot structure in a highly sensitive manner. We now investigate whether d_f is altered by stage of lung cancer, and if this is associated with mortality.

Methods: Blood samples were taken from 83 consecutive patients who attended clinic on diagnosis of primary lung cancer, and d_f measured immediately on whole blood. Standard laboratory markers (PT and APTT) were also measured. Results were compared by t-test, and $P < 0.05$ considered significant.

Results: Significantly increased d_f was identified in patients with lung cancer compared to controls (1.76 vs 1.72, $P < 0.05$), and increased further in those with extensive compared to limited disease (1.79 vs 1.73, $P < 0.05$). d_f was significantly increased in patients who had died compared to those alive 12 months after diagnosis (1.79 vs 1.74, $P < 0.05$). No differences were observed in standard laboratory markers.

Conclusion: Increased d_f was observed in lung cancer patients, particularly those with extensive disease, indicating that these patients form pathological clots of altered structure. d_f was further increased in patients who died within 12 months, suggesting that d_f may be used to screen patients, allowing for intervention in those deemed to be at increased CAT risk. Therefore, d_f is a highly sensitive test for quantifying hypercoagulability and abnormal clot structure in lung cancer patients, potentially predicting clinical outcome.

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PO157-WED

Prediction of cancer associated thrombosis. prospective biomarker study

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Background: Venous Thromboembolism (VTE) is a leading cause of death among patients with cancer. Improving risk stratification among patients with cancer may allow better selection for VTE prophylaxis. Multiple potential biomarkers have been suggested as potential Cancer-VTE predictors. There is however a paucity of data exploring the combined effect of novel biomarkers in a single cohort.

Aims: We sought to evaluate novel and validated cancer associated thrombosis biomarkers in a single cohort.

Methods: We designed a prospective sample collection, retrospective blinded evaluation, that was approved by the local IRB. We included outpatient adults with solid tumors planning to start chemotherapy. Patients who were pregnant, hospitalized within the prior 2 weeks, or had an acute VTE were excluded. Blood samples for biomarker analysis [D Dimer (dd), Protein C (pc), Factor VIII (fviii), thrombin-activatable fibrinolysis inhibitor, fibrin monomers] were collected prior to chemotherapy. VTE and death were periodically adjudicated. Biomarkers were categorized by quartiles. SAS 9.2 was used for data analysis with death as competing event.

Results: A total of 234 patients were analyzed: 60 years (median), 72% female, 22% low Khorana risk, 10.4 months follow up. A total of 30 (13%) patients developed VTE and 32 (14%) died. In the univariate biomarker analysis $dd > 1.5 \mu\text{g mL}^{-1}$ [Hazard Ratio, 95% Confidence Interval, P value] (2.7, 1.1-6.8, 0.03), $pc < 119 \mu\text{g mL}^{-1}$ (3.1, 1.5-6.7, < 0.01) and $fviii > 262 \text{ IU/dL}$ (4.5, 2.1-9.6, < 0.001) were associated with incident VTE. Results remained significant when adjusting for Khorana score, or functional status, or metastatic disease. When Khorana score and the three biomarkers were in the model, pc (2.6, 1.2-5.6, 0.01) and $fviii$ (2.9, 1.2-6.8, 0.01) remained strongly associated with VTE.

Conclusion: High D Dimer, Factor VIII as well as low Protein C levels are predictors of cancer associated VTE and can provide added stratification value when used in combination.

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PO158-WED

Changes in coagulation in the post operative period of abdominal cancer surgery

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Background: The main treatment of various cancers is surgical requiring that the coagulation process is operative and inflammatory conditions are controlled.

Aims: Evaluate cytokines, clotting factors, anticoagulants and fibrinolysis marker, both pre and post operative.

Methods: We evaluated 20 patients with gastrointestinal cancer undergoing surgical procedures. Before and after (12 h, 2 days and 5 days) surgery, blood was collected for the determination of coagulation components as well as inflammatory and anti inflammatory compounds such as interleukin (2,4, 6,10,17), tumor necrosis factor and interferon.

Results: There was a change of pro coagulant factors concentration, as compared with preoperative values: at 12 h, factor VIII and von Willebrand increases, whereas factor VII and X decrease. At 2 days there is an additional increase of fibrinogen. On the fifth day, fibrinogen, factor VIII and von Willebrand are elevated, Factor VII starts to normalize and factor XIII decreases to lower levels. Fibrinogen, Factor VIII and von Willebrand activity reached levels considered thrombogenic after 12 h, whereas factors VII, X and XIII decreased, reaching in some occasions levels below normal. Inhibitors of coagulation, protein C and free S were reduced on the second day and D-dimer increased at 12 h. Only IL-6 had detectable levels. Initially there was a progressive increase that was followed by a reduction initiated on the second day. This cytokine, despite having a pro inflammatory behavior, interacts reversely with fibrinogen and von Willebrand activity.

Conclusion: There is interplay between various components of the clotting system. Patients develop an increase of factors responsible for clotting, that is accompanied by a reduction of some coagulation inhibitors, but these changes are equilibrated by the reduction of some procoagulant factors. Interleukin 6 was the only cytokine consistently detected and may interfere significantly on coagulation.

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PO159-WED

Factor V leiden mutation and high FVIII are associated with an increased risk of VTE in women with breast cancer during adjuvant tamoxifen – results from a prospective, single center, case control study

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Background: Estimates of the risk ratio of tamoxifen-associated venous thromboembolism (VTE) in breast cancer patients range from 2.4 to 7.1. The occurrence of thrombosis in patients with breast cancer complicates the clinical condition and causes a change of treatment.

Aims: Our study was conducted in order to investigate the influence of patient-related risk factors for thrombosis development in breast cancer patients whose treatment included adjuvant tamoxifen.

Methods: The prospective, single center, case control study included 150 breast cancer women, 50 who developed venous thrombosis during adjuvant tamoxifen and 100 without thrombosis, as a control group. Patient-related risk factors such as: age, body mass index, previous VTE, varicose veins, concomitant diseases, the presence of prothrombotic mutations (FV Leiden, FII G20210A) and FVIII activity were evaluated in both groups.

Results: In respect of prothrombotic mutations, the FV Leiden mutation was present in a higher number of women from the VTE group (10/50 vs 7/100; $P = 0.020$). Additionally, FVIII activity was significantly higher in the VTE group; median (IQR), of 1.79 (0.69) vs 1.45 (0.55); $P < 0.001$ and more women in this group (24/50 vs 34/100) had

increased FVIII activity; $P = 0.020$. In those women with FVIII $> 1.5 \text{ IU mL}^{-1}$, who were carriers of prothrombotic mutations, an OR of 3.76 (CI 95% 1.276-11.096; $P = 0.016$) was obtained for VTE.

Conclusion: The results of our study showed that the factor V Leiden mutation and high FVIII are associated with an increased risk of VTE in women with breast cancer during adjuvant tamoxifen.

Disclosure of Interest: None Declared.

PO161-WED

Increasing platelets and other measures enhancing hemostasis are both important to prevent bleeding episodes when thrombocytopenic patients requiring antithrombotic therapy – a review of the medical literature

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Background: Patients with hypo-proliferative thrombocytopenia or immune-thrombocytopenia (ITP) may require anticoagulant for thromboembolism secondary to concurrent illness, putting them at a high risk of bleeding.

Aims: To perform a comprehensive review and summary of the literature published from 1974 till May 2014.

Methods: A literature search was conducted using the Ovid MEDLINE® database from Jan 1974 to May 2014 with the keyword of “thrombocytopeni*”. The search was limited to “case reports”, “case series” or “letters” written in English. Only publications on thrombocytopenia due to marrow dysfunction or ITP were included. Apart from demographic data and past medical history, the degree of thrombocytopenia during therapy, the details of antithrombotic treatment(s) and the clinical manifestations of bleeding were extracted to analyze the association between intervention strategies and bleeding outcomes with Fisher’s exact test. A P value of 0.05 was considered significant.

Results: The initial search strategy found 12,072 articles, among which 42 were included for data extraction. The cross-references were scanned for missing publications in our list. Those without information of antithrombotic treatment ($n = 2$) or bleeding outcome ($n = 1$) were omitted and three reports on thrombolysis only were excluded.

There were 32 hypo-proliferative thrombocytopenic patients requiring anticoagulants when platelets were below $50 \times 10^9/\text{L}$. Both platelet transfusion and measures promoting hemostasis significantly avoided bleeds. For 27 ITP patients requiring 28 brief antithrombotic therapies during invasive procedures such as coronary angioplasty, bleeding could possibly be prevented by concurrent therapy to increase platelets or other hemostatic measures, with borderline significant P values.

Conclusion: Literature reporting the experience of managing thrombocytopenic patients who required antithrombotic therapy suggests that, measures promoting better hemostasis are as important as increasing platelets to avoid bleeding complications.

Disclosure of Interest: None Declared.

PO162-WED

Pancreatic cancer patients express elevated FVIII levels and metastasis-associated fibrin turnover prior to their diagnosisVarkila N¹, Haglund C², Seppänen H² and Lassila R³¹University of Helsinki; ²Department of Gastroenterological Surgery; ³Coagulation Disorders Unit, Department of Hematology, Comprehensive Cancer Center, Helsinki University Hospital and University of Helsinki, Helsinki, Finland**Background:** Pancreatic ductaladenocarcinoma (PDAC) increases venous thromboembolism by 7-fold. The risk associates with Khorana score, which does not disclose the effects of cancer itself prior to oncological treatments (OT).**Aims:** To evaluate associations between cancer stage, risk scores and preoperative biomarkers.**Methods:** We enclosed (2010–14) 100 consecutively operated PDAC patients and 13 patients operated for benign intraductal papillary mucinous neoplasm (IPMN). Blood cell count and coagulation, inflammation and tumor markers were analyzed 1–3 days prior to surgery. Patients were divided into five groups: 1A: local PDAC no OT (n = 58), 1B: local PDAC with OT (n = 17), 2A: metastasized PDAC no OT (n = 12), 2B: metastasized PDAC with OT (n = 13) and 3: IPMN (n = 13).**Results:** CA 19-9 was higher in native local PDAC (1A) and metastasized PDAC with OT (2B) compared with IPMN (3) ($P < 0.05$). Median CA 19-9 was 156 kU L⁻¹ (2–11,935 kU L⁻¹) in local PDAC (1A), 1093 kU L⁻¹ (7–9186 kU L⁻¹) in metastatic PDAC with OT (2B) and 8 kU L⁻¹ in IPMN (3) ($P < 0.05$ 3 vs 1A and 2B). Mean FVIII:C was clearly higher in PDAC vs IPMN, 222% (92–381%, SEM 9.75) in 1A, 215% in 1B, 274% in 2A, 231% in 2B, but only 158% in 3 ($P < 0.002$ 3 vs 1A, 2A and 2B). D-dimer was higher both in metastasized PDAC with OT and native local PDAC vs. IPMN. Median D-dimer was 0.30 mg L⁻¹ (0.09–4.5 mg L⁻¹) in 1A, 0.90 mg L⁻¹ (0.19–4.5 mg L⁻¹) in 2B, but only in 0.19 mg L⁻¹ (0.09–1.5 mg L⁻¹) group 3 ($P < 0.002$ 3 vs 1A and 2B). CA 19-9 and D-dimer correlated in native PDAC (1A $r = 0.38$, $P = 0.004$) and especially metastasized PDAC (2A $r = 0.98$, $P < 0.001$). Khorana risk score did not correlate within the groups, and neither did other biomarkers differ.**Conclusion:** Khorana risk score did not associate with cancer stage or OT in this setting. CA 19-9 revealed native local PDAC and metastatic PDAC with OT vs. IPMN. High preoperative FVIII:C predicted PDAC with and without metastasis. Fibrin turnover in form of elevated D-dimer associated with local and especially metastatic PDAC with OT, and with CA 19-9.**Disclosure of Interest:** None Declared.

PO163-WED

Variations of plasma levels of TFPI and activated FVII-antithrombin complex in cancer patients: the increase of TFPI as a major determinant of thrombin generationVan Dreden P¹, Doubre H², François D³, Adam M¹, Grusse M¹ and Vasse M³¹Clinical research, Diagnostica Stago, Gennevilliers;²Pneumology; ³Clinical Biology, Hospital Foch, Suresnes, France**Background:** Cancer patients are at increased risk of thrombosis, but the physiopathological mechanism is still unclear. The activity of Tissue Factor (TF), the main activator of coagulation, is regulated by the TF Pathway Inhibitor (TFPI). Activated Factor VII can be inhibited by antithrombin (AT), leading to the formation of FVIIa-AT complexes.**Aims:** We analysed the activation of the extrinsic pathway in cancer patients and quantified free TFPI and FVIIa-AT complexes.**Methods:** 119 patients with newly diagnosed cancer of the lung (n = 28), prostate (n = 50), bladder (n = 18) and digestive tract

(n = 22) and 30 healthy controls (CT) were studied. Free TFPI (Asserachrom Free TFPI, Asserachrom VIIa-AT, Diagnostica Stago) were determined. Thrombin generation (TG) was measured by CAT assay (5 pM of TF).

Results: TFPI levels were significantly increased in cancer patients [median 17.8; range (R): 7–58 µg L⁻¹] vs. CT (median: 9.1; (R): 5.9–13.5, $P < 0.0001$), and this was observed for each subgroup of cancer. FVIIa-AT complexes were significantly decreased only in patients with digestive cancers [median 56 pM; (R): 29.8–62.5 vs. 97.6; (R): 68.9–132.2 in CT, $P < 0.0001$]. Considering the TNM staging (*WHO classification*, 2009) of tumours, we observed that plasma TFPI levels increased significantly with tumour size and the presence of metastasis, whereas FVII-AT complexes levels decreased with the size of the tumour and the presence of lymph nodes. There was a significant decrease of TG in cancer patients vs. CT. The lag-time and time to peak were positively correlated to plasma TFPI levels, whereas endogenous thrombin potential was inversely correlated.**Conclusion:** The decrease of FVIIa-AT complexes observed in patients with digestive cancer could be due to liver dysfunction and the elevation of TFPI could contribute to decrease the thrombotic risk of cancer patients. It could be of interest to analyze whether patients with a low plasma level of TFPI are at higher risk of thrombosis than the others.**Disclosure of Interest:** None Declared.

PO164-WED

Protein c levels are associated with mortality in patients with advanced cancerWilts D¹, Hutten BA², Meijers JC^{3,4}, Spek CA⁵, Büller HR³ and Kamphuisen PW¹¹Department of Vascular Medicine, University Medical CenterGroningen, Groningen; ²Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Amsterdam Medical Centre;³Department of Vascular Medicine, Amsterdam Medical Center;⁴Department of Plasma Proteins, Sanquin Blood Supply; ⁵Center for Experimental and Molecular Medicine, Amsterdam Medical Center, Amsterdam, Netherlands**Background:** In cancer, tumor progression and metastasis are promoted by prohemostatic activity. Protein C (PC) is involved in hemostasis, inflammation and signal transduction, and has a protective effect on the endothelial barrier. In mice, administration of activated PC reduced experimental metastasis. It is unclear whether PC level is associated with mortality in patients with cancer.**Aims:** To assess the relation between PC level and survival in patients with advanced cancer.**Methods:** A multicenter, randomized, open-label study was performed in 11 countries between May 2006 and August 2008 (Inpact study, van Doormaal et al., JCO 2011). Patients (n = 503) with hormone-refractory prostate cancer, non-small cell lung cancer stage IIIB and locally advanced pancreatic cancer were randomized to receive nadroparin or placebo for 6–46 weeks following a specific schedule. Patients were followed till death or the end of the study in May 2009. PC activity levels were measured at baseline and categorized in tertiles. The association between PC level and mortality was evaluated with Cox proportional hazard models, adjustments were made by multivariate Cox proportional hazard models.**Results:** PC level could be measured in 479 (95%) patients (tertiles: < 97, 97–120 and > 120 IU dL⁻¹). Mean age was 65 ± 10 years; 87 (18%) were female; and 161 patients had lung cancer, 125 pancreatic cancer and 191 prostate cancer. In two patients cancer type was missing. During median follow-up of 10.5 months, 291 (61%) patients died. Median PC level was 107 IU dL⁻¹ (IQR 92–129). There was a clear inverse relation between PC level and mortality (P for trend 0.036). In the lowest tertile, mortality was 64%, in the middle and high tertile 61% and 55%, respectively. Compared to the highest tertile, the

lowest tertile was associated with a HR on mortality of 1.36 (95% CI 1.02-1.80). Adjustment for age, gender and nadroparin use did not affect this association.

Conclusion: Lower PC levels are associated with increased mortality in patients with advanced cancer.

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PO165-WED

L-asparaginase lowers plasma antithrombin and mannan-binding-lectin levels: impact on thrombotic and infectious events in children with acute lymphoblastic leukemia

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Background: L-asparaginase, a key therapeutic agent in the management of patients with acute lymphoblastic leukemia (ALL), dramatically impairs hepatic protein synthesis.

Aims: We investigated the effects of prolonged exposure to L-asparaginase on antithrombin (AT), fibrinogen and mannan-binding lectin (MBL) levels, and on the occurrence of thrombotic events (TE) and febrile neutropenia episodes (FN) in pediatric patients.

Methods: Protein levels were measured in 97 children during 30 weeks of chemotherapy with L-asparaginase and up to 1 year following remission. TE and FN episodes were recorded during this period.

Results: Median AT level decreased from 0.96 IU mL⁻¹ prior to treatment (range: 0.69-1.38) to 0.55 IU mL⁻¹ (0.37-0.76) during therapy. Fibrinogen and MBL decreased from 3.18 g L⁻¹ (1.29-7.28) and 1,177 ng mL⁻¹ (57-5343) to 1.56 g L⁻¹ (0.84-2.13) and 193 ng mL⁻¹ (57-544), respectively. All three proteins had recovered 1-4 weeks after L-asparaginase cessation. TE were reported in 22 (23%) patients. Of these, 11 occurred after a median of 10 administrations of L-asparaginase. Fifty-one FN were associated with infections, of which 36 occurred during treatment with L-asparaginase. Patients with low levels of MBL at diagnosis were at higher risk of FN associated with infections (RR=1.59, 95% CI: 1.026-2.474). Both AT and MBL decreases were moderately correlated with fibrinogen ($r = 0.51$ and 0.58 respectively). **Conclusion:** Children with ALL are exposed to significant decrease in AT, fibrinogen and MBL levels, and concomitant increased risk of thrombosis and FN with infection during L-asparaginase treatment. Measuring plasma levels of these liver-derived proteins could help predict the occurrence of adverse events.

Disclosure of Interest: None Declared.

PO166-WED

Longitudinal analysis of hemostatic biomarkers in cancer patients during anti-tumor treatment

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Background: Biomarkers of hemostasis are known to have an impact on occurrence of venous thromboembolism (VTE) and survival in cancer patients.

Aims: As there are almost no data regarding longitudinal changes, we aimed to evaluate selected biomarkers during the course of disease.

Methods: Patients with cancer of the brain ($n = 39$), lung ($n = 41$), colon ($n = 15$) or pancreas ($n = 17$) were included before initiation of anti-cancer therapy. Blood samples for determination of factor VIII (FVIII), peak height thrombin, D-dimer, prothrombin fragment F1 + 2, fibrinogen and soluble (s) P-selectin were drawn on a monthly basis. Study end points were death, loss of follow-up or completion of the study period (max. 250 days).

Results: Overall, 546 blood samples of 112 patients were analysed. D-dimer ($P = 0.02$) and sP-selectin ($P = 0.05$) levels were significantly higher in patients with distant metastasis compared to those without. Patients, who achieved complete remission under chemo- and radiotherapy, showed significantly lower levels of prothrombin fragment F1 + 2 ($P = 0.02$), D-dimer ($P < 0.01$) and fibrinogen ($P = 0.01$) compared to patients without complete tumor response. Peak height thrombin levels showed a decrease by time in all tumor types. Levels of biomarkers behaved differently in the various tumor types. Patients, who developed VTE, showed increasing levels of FVIII, sP-selectin and D-dimer, but the individual course was highly variable. Regarding survival, increased FVIII, sP-selectin and D-dimer levels during the time of disease were associated with higher mortality.

Conclusion: Hemostatic biomarkers may reflect the disease state, prognosis and risk of VTE, not only at diagnosis, but also during the course of anti-neoplastic treatment. Due to the highly variable course of the parameters, an improved risk prediction by longitudinal determination of biomarkers cannot be expected.

Disclosure of Interest: None Declared.

PO167-WED

Microvesicles are a potential biomarker for thrombosis and DIC in leukemic patients: a cross-sectional study

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Background: Thrombosis induced by a hypercoagulable state is a common complication of patients with malignancies. Our previous studies suggest leukemic cells shed procoagulant extracellular vesicles (EVs) and this EVs procoagulant activity (PCA) may play a major role in venous thromboembolism or disseminated intravascular coagulation (DIC) in acute leukemia.

Aims: The aim is to study the impact of EVs from leukemic patients on thrombin generation and if EVs-PCA could be a potential biomarker for thrombotic complication in patients with acute leukemia.

Methods: Blood samples from 38 patients with acute leukemia newly diagnosed were obtained at Day-0 (before treatment), D-3 and D-7 (3 and 7 days after treatment). Extracellular vesicles were isolated and concentrated by ultracentrifugation. EVs-PCA and EVs-tissue factor

activity was measured by a thrombin generation assay and a commercial bio-immunoassay (Zymuphen MP-TF®), respectively.

Results: Among acute leukemic patients, three patients had an increased EVs PCA at D-0 and two of them developed a thrombotic event. The remaining patients without thrombotic events did not show an increased EVs-PCA. One patient had an increased extracellular vesicles procoagulant activity at D-3 and developed a DIC at D-5. Three patients had an increased EVs-tissue factor activity ($> 2 \text{ pg mL}^{-1}$), among whom two developed a thrombotic event and one had haemorrhage. A plasma from a patient with induced DIC had no increased extracellular vesicles tissue factor activity from D-0 to D-7 ($< 2 \text{ pg mL}^{-1}$).

Conclusion: The results suggest a strong association between thrombotic events and extracellular vesicles procoagulant activity. Moreover, extracellular vesicles procoagulant activity has a better predictive value for venous thromboembolism and DIC in patients with acute leukemia than D-Dimer, leucocyte and platelet count, fibrinogen and coagulation time (thrombin time and activated partial thromboplastin time).

Disclosure of Interest: None Declared.

PO168-WED

Genetic variants in 3'-UTRS of methylenetetrahydrofolate reductase (MTHFR) predict colorectal cancer susceptibility in Koreans

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Background: MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate; the latter is the methyl donor for the conversion of homocysteine (Hcy) to methionine, whereas the former, and its derivatives, are essential cofactors for both thymidylate and *de novo* purine synthesis. This conversion is indispensable for the thrombotic balance *in vivo*. Decreased MTHFR activity and expression lead to an accumulation of Hcy and/or deficiency of FA. Recent studies have shown some clinical impacts of polymorphisms in the 3'-UTR of certain genes, which may potentially bind to specific microRNAs (miRNAs) in various cancers. However, variants in the MTHFR 3'-UTR have not been extensively studied.

Aims: The aim of this study was to investigate the association of four MTHFR polymorphisms, 2572C>A [rs4846049], 4869C>G [rs1537514], 5488C>T [rs3737967], and 6685T>C [rs4846048] with colorectal cancer (CRC) in Koreans.

Methods: The genotyping of MTHFR 3'-UTR polymorphisms was performed by polymerase chain reaction-restriction fragment length polymorphism analysis or TaqMan allelic discrimination assays. A total of 850 participants (450 CRC patients and 400 controls) were enrolled in the study.

Results: We found that MTHFR 2572C>A, 4869C>G, and 5488C>T genotypes were substantially associated with CRC susceptibility. Of the potentially susceptible polymorphisms, MTHFR 2572C>A was associated with increased homocysteine and decreased folate levels in the plasma based on MTHFR 677CC.

Conclusion: Our study provides the evidences for 3'-UTR variants in MTHFR gene as potential biomarkers for use in CRC prevention.

Disclosure of Interest: None Declared.

PO169-WED

Significance of haemostatic parameters in patients with urinary cancer

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Background: Multiple studies demonstrated a key role of angiogenesis, thrombosis and fibrinolysis in tumour invasion and metastasis.

Aims: To study prospectively hemostatic parameters in patients diagnosed with urinary cancer and correlation of these parameters at the time of diagnosis with the type of disease, grade, stage and prognosis.

Methods: Sample consisted of 58 subjects in the control group and 147 with Ca (type of cancer: renal n = 44, prostate n = 56, bladder n = 47). Samples were collected with standard procedures and examined with screening tests (PT, aPTT) and fibrinogen levels, D-Dimers, prothrombin fragments F1,2 and TAT complexes. Statistical analysis was performed using SPSS Statistical software (version 19.0).

Results: Higher levels of Fibrinogen and D-Dimers were found in the cancer group. Subgroup comparisons showed higher levels of aPTT in the renal Ca group ($P = 0.039$). Fibrinogen was higher in all Ca groups ($P < 0.05$), while D-Dimers were higher in renal ($P = 0.004$) and prostate ($P = 0.005$) cancer. Tumor grade was positively correlated with PT, while increased values of D-Dimers and F1 + 2 were associated with greater TNM stage. A positive correlation was found between Fuhrman grade and aPTT and Fibrinogen. Higher D-Dimers values and F1 + 2 were found in cancer patients with advanced TNM stage (2-4) as compared with TNM stage (0-1). When multiple logistic regression analysis conducted it was found that Fibrinogen was an independent factor for TNM stage ≥ 2 (for 100 mg increase): 1.33, 95% CI: 1.00-1.76, $P = 0.047$. Multiple logistic regression analysis for having high grade indicated that elevated levels of D-Dimers were independently associated with greater likelihood of having high grade cancer (OR:2.33, 95% CI: 1.09-5.00, $P = 0.030$).

Conclusion: Plasma levels of hemostatic parameters are altered in patients with urinary cancer and there is a possible correlation between stage and grade. Further study is needed to elucidate the clinical significance among patients with urinary cancer.

Disclosure of Interest: None Declared.

PO170-WED

Evaluation of thromboelastometry parameters as predictive markers for sinusoidal obstruction syndrome in patients undergoing allogeneic stem cell transplantation for acute leukemia

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Background: Hepatic sinusoidal obstruction syndrome (SOS, also named veno-occlusive disease) is a serious complication of allogeneic stem cell transplantation (HSCT). Early identification of patients at risk of SOS may probably influence the outcome and reduce mortality.

Aims: We evaluate the coagulation abnormalities in patients undergoing allogeneic HSCT for acute leukemia by rotation thromboelastometry (ROTEM) with respect to SOS occurrence.

Methods: A total of 27 patients (median age 40, range 19-63) with acute leukemia, undergoing allogeneic HSCT after myeloablative (n = 16) or reduced-intensity conditioning regimen (n = 11), were analysed. ROTEM assays (INTEM, EXTEM, FIBTEM, APTEM) were performed before the conditioning regimen start (day -10), on the

day of stem cell infusion (day 0) and on day +14 and +28 after HSCT. The diagnosis of SOS was based on Baltimore criteria.

Results: Seven patients developed SOS (26%) during observation. None of them developed a severe SOS or multiorgan failure. Weight gain and jaundice were the most usual manifestation. Major coagulation abnormalities were observed on day 0.

On day +14, patients with SOS had statistically significant longer EXTEM-CT (clot time, 69.5 ± 6.39 vs. 52 ± 3.42) and FIBTEM-CT (69.5 ± 22.7 vs. 50.8 ± 14.3 s) compared to patients without SOS ($P < 0.014$). The ROC analysis indicated the cutoff value of 69 s in EXTEM-CT (AUC=0.90; 95%CI 0.75-1.0, SE=0.076) and 102 s in FIBTEM-CT (AUC=0.82; 95%CI 0.61-1.0, SE=0.104) for the prediction of SOS in patients undergoing HSCT on day +14. Besides, no significant differences in clinical characteristic and other thromboelastometry results were observed between patients with or without SOS.

Conclusion: Our results suggest that specific thromboelastometric profile including delay in activation of hemostasis in the EXTEM and FIBTEM assay may predict sinusoidal obstruction syndrome occurrence in patients undergoing allogeneic HSCT for acute leukemia. The utility of ROTEM assays should be clarified in further prospective studies.

Disclosure of Interest: None Declared.

PO171-WED

Cancer inducing netosis promotes widespread arterial thrombosis presenting as ischemic stroke with troponin elevation

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Background: Troponin elevation in ischemic stroke is associated with a higher mortality, and the reasons are not fully understood. Neutrophils releasing chromatin as neutrophil extracellular traps (NETs) have been linked to ischemic stroke and coronary artery disease. NETs can be induced by cancer, and are pro-thrombotic. Cancer is a well-established risk factor for venous thromboembolism, but its association with arterial thrombosis is not clear.

Aims: To determine whether troponin elevation in stroke patients could be the result of a NET-induced procoagulant state leading to cerebral and myocardial ischemia.

Methods: Clinical assessment, plasma analyses and autopsy (in cases of in-hospital mortality) were performed on 12 patients with ischemic stroke and elevated troponin T (TnT) and 20 patients with ischemic stroke and normal TnT.

Results: A four-fold increase of the NET-specific biomarker citrullinated histone H3 (H3Cit) was found in patients with TnT elevation ($P < 0.002$). Autopsy in three of these patients showed normal coronary arteries but widespread myocardial microthrombosis with H3Cit in thrombi. Patients with TnT elevation also had a surprisingly higher prevalence of cancer (58% vs 5%, $P < 0.003$), some of which were diagnosed post-mortem. Plasma analyses in cancer patients showed higher H3Cit ($P < 0.001$) as well as an increase in leukocyte count and granulocyte colony-stimulating factor, shown to prime neutrophils towards NETosis. The presence of NET biomarkers correlated positively with thrombin-antithrombin complex

($P < 0.001$) and soluble P-selectin ($p < 0.001$), linking NETosis to the pro-thrombotic state.

Conclusion: We show cancer-associated thrombosis presenting as ischemic stroke with TnT elevation. We link this to NETosis, identifying biomarkers which could aid in revealing cancer in patients with widespread arterial thrombosis. These biomarkers also reveal possible new targets for antithrombotic treatment.

Disclosure of Interest: None Declared.

PO172-WED

Relevance of D-dimer and IL-6 levels among patients with cancer and incidental pulmonary embolism

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Background: Patients with cancer and incidental pulmonary embolism (IPE) found on routine staging scans are frequently symptomatic with fatigue and shortness of breath.

Aims:

- 1) To examine whether biologic correlates of thrombosis are similar between oncology patients with IPE and suspected PE (SPE), or are associated with clinical characteristics and outcomes.
- 2) To identify an association between d-dimer or IL-6 levels among cancer patients with IPE correlate with the presence of reported symptoms
- 3) To compare outcomes of cancer patients with IPE or SPE treated with low molecular weight heparin

Methods: We conducted a retrospective analysis of a single-arm prospective phase II study evaluating the efficacy, safety and impact on biologic correlates of six months of tinzaparin in patients with cancer diagnosed with venous thromboembolism (VTE). Associations between D-dimer and IL-6 levels and signs/symptoms, PE location, VTE recurrence, and overall survival (OS) were evaluated in IPE and SPE patients. Cutoff levels for elevated D-dimer and IL-6 levels were set at the median of the study population.

Results: Of 66 patients with PE, 76% had IPE. D-dimer and IL-6 levels, PE distribution, VTE recurrence and OS were similar in IPE and SPE patients. Higher D-dimer levels were detected in patients with fatigue ($3,796 \text{ ng mL}^{-1}$ v $1,641 \text{ ng mL}^{-1}$; $P = 0.006$) and PE more proximal than the subsegmental artery ($2,946$ v $1,420 \text{ ng mL}^{-1}$; $P = 0.030$). Elevated D-dimer (26% v 11%; $P = 0.034$) or IL-6 (35% v 8%; $P = 0.039$) levels were associated with a higher cumulative probability of VTE recurrence at 12 months in multivariate analysis.

Conclusion: Oncology patients with IPE and SPE have comparable D-dimer and IL-6 levels, thrombus burden, and outcomes. Elevated pre-treatment D-dimer and IL-6 may help identify patients with cancer and PE at increased risk for VTE recurrence and they appear to correlate with the presence of reported symptoms, particularly fatigue.

Disclosure of Interest: None Declared.

PO173-WED

Diagnostic approach to rare localisation thrombosis in cancer patients

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Background: Thrombosis of rare localizations: hepatic vein thrombosis, splenic, mesenteric, ovarian veins, brain, portal vein are life-threatening disorders, which often goes unrecognized. The most frequently atypical localizations develop thrombosis in patients with inherited defects of hemostasis - genetic thrombophilia. Rare localizations of thrombosis often accompanied acquired abnormalities of hemostasis, which include antiphospholipid syndrome, hypercoagulation in oncology

Aims: The detection of thrombosis rare localizations required screening for genetic forms of thrombophilia and antiphospholipid syndrome and timely appointment of anticoagulant therapy

Methods: Since 2006 we have observed 1214 patients with gynecological cancer.

Results: In 10 cases we have observed unusual localization thrombosis: Hepatic vein thrombosis (Budd-Chiari syndrome) – in one case, Splenic vein thrombosis – two cases, renal vein thrombosis – one case, thrombosis of retinal artery and vein – three cases, Cerebral venous thrombosis – one case, mesenteric thrombosis – two cases, one of them has lead to death in early postsurgery period.

APA circulation has been found in all cases: antibodies to B2Gp1a, antibodies to Annexin V and antibodies to prothrombin.

The factor FV Leiden homozygous mutation was found nine patients, except one patient with retinal vein thrombosis, the homozygous MTHFR mutation has been found in nine patients, heterozygous in 2; prothrombin mutation in 9; PAI-1 polymorphism in all 10 cases, platelets glycoproteins polymorphism in seven cases.

In 56 years patient with ovarian cancer despite of anticoagulation therapy with LMWH has been found mesenteric thrombosis in 2 day after surgery. In addition to the above mentioned mutations it was found ADAMTS13 gene mutation.

Conclusion: Presence of multigene forms of genetic thrombophilia and APA-circulation increase risk of rare localizations thrombotic complications in cancer patients, therefore such patients required in intensive permanent preventive maintenance with use of LMWH

Disclosure of Interest: None Declared.

PO174-WED

Clinical significance of circulating tissue factor (TF) in women with suspected ovarian cancerClaussen C¹, Rausch AV², Spath B¹, Lezius S³, Amirkhosravi A⁴, Davila M⁴, Francis JL⁴, Bokemeyer C¹, Jänicke F², Mahner S² and Langer F¹

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Background: The interplay between malignancy, inflammation, and coagulation is well established. It is currently unclear, however, whether preoperative assessment of circulating TF provides valuable additional information in women presenting with ovarian masses of unknown etiology.

Aims: To evaluate circulating TF as a diagnostic tool in suspected ovarian cancer.

Methods: In a prospective single-center cohort study, we measured preoperative plasma levels of D-dimer, TF antigen, microparticle-associated TF-specific procoagulant activity (MP TF PCA), and soluble P-

selectin (sCD62P) in 40 women with a final diagnosis of ovarian cancer in comparison to 15 women with benign tumors, all of whom underwent exploratory surgery, and 34 healthy females.

Results: D-Dimer, MP TF PCA, and sCD62P, but not TF antigen and CA125, were significantly increased in cancer patients, while levels of hemostatic markers were similar in patients with benign tumors and healthy controls. Plasma depletion of MPs did not significantly alter TF antigen levels, as measured by ELISA, suggesting that this assay predominantly captured a soluble TF variant. In cancer patients, only D-Dimer and CA125 correlated with the FIGO stage. Abnormal levels of D-dimer had the highest sensitivity (93%), while increased MP TF PCA had the highest specificity (78%) for the diagnosis of cancer. Peri-operative venous thromboembolism (VTE) occurred in 16% of cancer patients and was associated with an advanced FIGO stage, while sCD62P and plasma fibrinogen, an acute-phase reactant, were correlated with the Khorana score, a validated VTE risk assessment tool in ambulatory cancer patients.

Conclusion: While D-dimer, by reflecting the overall impact of the cancer on the hemostatic system, is correlated with tumor burden, shedding of MP TF PCA may provide complementary information relevant to malignant transformation and cancer biology. In this regard, technically demanding analysis of MP TF PCA cannot be replaced by measurement of plasma TF antigen.

Disclosure of Interest: None Declared.

PO175-WED

Rivaroxaban in patients with VTE and cancer: a single center experience

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Background: Patients (pts) with cancer are a very high risk of venous thromboembolism (VTE). The overall survival in cancer pts with VTE is shorter than cancer pts without VTE, while the risk of recurrence, bleeding complications or morbidity is increased. Treatment and prophylaxis of VTE are needed to improve clinical outcome, but unlike conventional therapy with heparins or fondaparinux, use of direct oral anticoagulants (DOACs) is still debated, in fact only few cancer pts were included in clinical trials on DOACs

Aims: To evaluate the efficacy and safety of rivaroxaban in cancer pts with VTE.

Methods: Consecutive cancer pts, > 18 years, males and females, with deep vein thrombosis (DVT) or pulmonary embolism (PE), treated with rivaroxaban from Sep 2013 to Dec 2014 are considered in our study. Rivaroxaban was administered for acute or long term treatment or for recurrence prevention of VTE in pts with cancer at several sites. Follow-up (FU) was established at 3, 6 and 12 months from the start of treatment with DOAC

Results: 291 consecutive pts were treated for VTE in our center, among these 17.9% were cancer pts, 50% males, mean age 67 years (range 39–90). 48.1% of cancer pts had DVT, 32.7% PE and 19.2% DVT/PE. Breast cancer was found in 26.8% of pts, genitourinary in 19.2%, colorectal in 15.4%, gastric in 13.5% and lung in 5.8%. Active cancer was present in 36.5% of cases, complete or partial remission in 42.5% and chronic, but stable disease in 21.2%. 27 pts were treated with chemotherapy, 4 pts with radiotherapy. Mean time in rivaroxaban was 5 months (range 1-14). During FU only one myocardial infarction occurred in the first two months of therapy in a patient with active colorectal cancer and acute coronary syndrome. No bleeding or other adverse events were reported

Conclusion: Use of DOACs in cancer pts with VTE are still debated and only few cases has been reported, but our small study showed that rivaroxaban was effective and safe than conventional parenteral therapy

Disclosure of Interest: None Declared.

PO176-WED

D-dimer plasma levels as a marker of pulmonary embolism in patients undergoing surgery for tumors of the central nervous system

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Background: Tumors of the central nervous system (CNS) are associated with an increased risk of pulmonary embolism (PE), particularly after surgery. In patients with CNS tumors, post-operative antithrombotic prophylaxis is challenging because of the concomitant risk of bleeding. Finding predictive PE markers after surgery will help to tailor antithrombotic prophylaxis in these patients. High D-dimer is a good predictor of venous thromboembolism (VTE).

Aims: To evaluate whether D-dimer plasma levels measured at the end of surgery are a predictive marker of early (within 7 days) PE in patients undergoing surgery for CNS tumors.

Methods: One hundred consecutive patients (median age 62y [IQR:49-71]) undergoing surgery for CNS tumors (55 meningioma and 45 glioblastoma) were included. D-dimer plasma levels were measured before and immediately after surgery. Demographic data were collected and lung perfusion scan was performed before and 2–7 days after surgery. PE was diagnosed in case of intermediate or high probability results of perfusion lung scan after surgery (with a normal preoperative scan) confirmed with CT scan.

Results: After surgery 21 patients (21%) developed PE, and median (IQR) D-dimer levels were 713 ng mL⁻¹ (355–1667), with a median increase of 440 ng mL⁻¹ (199–1306) compared to pre-operative levels. After adjustment for sex, age and type of tumor in a logistic regression model, the risk of PE linearly increased with post-surgery D-dimer levels (adjusted odds ratio 1.11 [95%CI:0.99–1.25] for every 500 ng mL⁻¹ increase). Patients with D-dimer levels above the median had a 3-fold increased risk of PE than those with levels below the median (adjusted odds ratio 2.82 [95%CI:0.78–10.2]).

Conclusion: D-dimer levels measured immediately after surgery for CNS tumors are a promising marker of early PE and might help to identify patients at high risk of PE, who may deserve a more aggressive antithrombotic prophylaxis.

Disclosure of Interest: None Declared.

PO177-WED

Tissue factor pathway inhibitor (TFPI) is a marker of tumor progression and prognosis in breast cancer

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Background: Hypercoagulability in malignancy encompasses increased thrombotic risk and involvement in cancer progression. Tissue factor (TF) and TF pathway inhibitor (TFPI) have shown tumor-promoting and suppressing effects, respectively.

Aims: We aimed to investigate the clinical relevance of TFPI and TF genetic and phenotypic diversity in breast cancer. In addition, we investigated if genetic variants beyond the *TFPI* gene could affect plasma TFPI levels.

Methods: Tumor mRNA expression (Agilent microarray), plasma protein levels of TF and free and total TFPI (ELISA), tagSNPs in *F3* (*TF*) (n = 6), *TFPI* (n = 18), *F5* (n = 23), *F10* (n = 15), *PROS1* (n = 7) (Sequenom MassARRAY), and clinicopathological characteristics and molecular subtypes (PAM50) were explored in 152 breast cancer patients. Survival analyses were performed in a merged breast cancer dataset (n = 1881).

Results: Patients with PR-negativity, larger tumors, lymph node metastases, and HER2 positivity showed increased expression of TFPIα or TFPIβ. In contrast, TFPI plasma levels decreased by increasing tumor size and triple negativity. TF mRNA expression was decreased in grade 3 tumors.

SNP haplotypes of *TFPI* were significantly associated with tumor size (OR=3.14), triple negativity (OR=2.4), lymph node spread (OR=3.34), and basal (OR=2.3) and luminal B tumors (OR=3.5). Several transcriptional/translational regulatory *TFPI* SNPs appeared, providing a possible mechanistic rationale for the variability in TFPI mRNA and plasma levels across clinical groups.

Tumor-expressed TFPIα and TFPIβ were associated with increased overall- and relapse free survival in all tumors and in lymph node positive patients.

None of the SNPs in *F5*, *F10*, or *PROS1* had any significant effect on plasma TFPI levels.

Conclusion: This study indicates that genetic and phenotypic variables of both TFPIα and TFPIβ are superior to TF as markers of cancer progression. The beneficial effect of tumor expressed TFPI on survival renders TFPI as a potential anticancer agent.

Disclosure of Interest: None Declared.

PO178-WED

Increased activated protein c resistance and D-dimer in breast cancer: phenotypic and genotypic determinants and association with tumor characteristics

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Background: Coagulopathy is a frequent phenomenon in cancer. We have previously observed acquired activated protein C resistance (APCR) and increased levels of D-dimer in breast cancer.

Aims: 1) Identify determinants of APCR (in factor V Leiden non-carriers) and D-dimer in breast cancer patients; 2) search for differences in APCR and D-dimer levels across clinical breast cancer subgroups.

Methods: APCR, D-dimer, endogenous thrombin potential (ETP), protein C, protein S, antithrombin (AT), fibrinogen, factor (F) VIII, FIX, FX, free tissue factor pathway inhibitor (TFPI), total TFPI, and von Willebrand factor (VWF) were determined in plasma of 366 treatment naive, surgical breast cancer patients.* The following clinicopathological tumor characteristics were included: estrogen- and progesterone receptor, human epidermal growth factor receptor 2, histological grade, tumor size, and lymph node spread.

*Signed a written consent. The study was approved by the Regional Ethics Committee.

Results: Multiple linear regression analysis including age and levels of the coagulation factors/inhibitors showed that the strongest determinants of APCR were fibrinogen ($r = -0.44$ $P < 0.001$), peak ETP ($r = 0.40$ $P < 0.001$), and protein S ($r = -0.35$ $P < 0.001$), whereas protein C ($r = 0.22$ $P = 0.002$), FIX ($r = 0.23$ $P = 0.014$), free TFPI, and lag time were moderate/weak determinants.

The strongest determinant of D-dimer levels were free TFPI ($r = 0.28$ $P < 0.001$) and AT ($r = -0.22$ $P = 0.001$), and weaker correlations were found for protein S and FX.

Neither APCR nor levels of D-dimer were associated with tumor characteristics.

Associations between SNPs in the phenotypic determinants, in addition to FV, and APCR/D-dimer will be presented.

Conclusion: Coagulation factors and inhibitors that contribute to explain APCR and D-dimer levels in breast cancer patients were demonstrated. However, APCR and D-dimer could not predict tumor aggressiveness in this cohort.

Disclosure of Interest: None Declared.

PO179-WED

Thrombin generation (TG) is downregulated by low molecular weight heparin (LMWH) in cancer patients with venous thromboembolism (VTE)

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Background: LMWH is recommended for initial and long-term anticoagulant therapy for cancer-associated VTE. However, the VTE recurrence rate with LMWH still remains significant (9-15%). It may be important to measure LMWH anticoagulant effect *in vivo* and possibly identify patients at highest risk of VTE recurrence

Aims: In a prospective cohort of cancer patients with VTE receiving LMWH nadroparin 200 UI kg⁻¹ once a day, we evaluated: 1. Whether Thrombin Generation (TG), a global hemostatic assay, is consistently downregulated by LMWH treatment; 2. How TG levels relate to microparticle (MP)-procoagulant activity (PCA) and D-dimer levels (HemosIL D-dimer); and 3. If any of these markers predicts for VTE recurrence

Methods: Cancer patients with acute VTE (n = 62) were enrolled. Plasma samples were obtained: at the thrombotic event, before LMWH (T0), at 1 month of LMWH therapy, and at discontinuation, i.e. 6 months after VTE. Patients were followed up to 9 months

Results: The most represented malignancies were colon, breast, lung, gastric cancers. VTE sites were: subclavian, femoro-popliteal, pulmonary, jugular, and brachial veins. At T0, patients had significantly higher both TG, MP-PCA, and D-dimer levels vs. controls. During LMWH, a significant reduction in TG potential occurred ($P < .05$) together with D-dimer only. After LMWH discontinuation, TG potential as well as D-Dimer levels rose back to the control levels. No variations were observed in MP-PCA. No VTE recurrences occurred; 29 patients (47%) died during follow-up for cancer. High D-dimer level at enrolment predicted for reduced survival rate (HR=3.69)

Conclusion: In this cohort, LMWH treatment was effective and safe. Without VTE recurrences, no predictive value for the biomarkers was established. However, D-dimer was for predictive for poor survival. Our results show that the global TG *in vivo* is down-regulated by LMWH, together with D-dimer. It is worth to define in a large trial the clinical utility of TG assay to monitor anticoagulation in cancer patients

Disclosure of Interest: None Declared.

PO180-WED

Hypercoagulability: an added side effect for androgen deprivation therapy in prostate cancer patients

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Background: Androgen deprivation therapy (ADT) is a common and effective treatment for prostate cancer (PCa). Several side effects have been reported in association with ADT: increased triglycerides and HDL, insulin resistance and cardiovascular morbidity and mortality. The effect of ADT on blood coagulability has not been systematically studied. A recent study from our group showed that 69% of PCa patients are hypercoagulable and this hypercoagulability is more pronounced in patients under ADT. More over, 32% of these patients developed one or more arterial or venous thromboembolic events over 12 months follow up period.

Aims: To investigate the effect of ADT on blood coagulation.

Methods: A prospective longitudinal study. Twenty two prostate cancer patients starting androgen deprivation therapy were followed up over 18 months. Global hemostasis was evaluated using Thromboelastography (TEG) prior to treatment and at 3 months intervals. Thirteen patients completed 1-3 assessments and nine completed 3-6 assessments. Descriptive statistics were used to evaluate the changes in TEG parameters and hypercoagulability was evaluated in comparison to an age-matched control group. Repeated measures ANOVA was used to evaluate four patients who had evaluations at the similar time points.

Results: Overall assessment of the 22 patients showed hypercoagulability indicated by 10% shortening of time to clot (R), 10% increase in rate of fibrin formation (α angle), 10% increase in clot strength (MA) and 62% increase in overall clotting index (CI) compared to the control. In 10 patients, 3 months after initial treatment, there was a 5% increase in MA and 14% in CI. In four patients, four consecutive assessments over 12 months period, showed an increase in MA(8%) and CI(30%). However, the repeated measures ANOVA showed no significance in any of the TEG parameters.

Conclusion: ADT appears to be associated with a hypercoagulable state that necessitates patient monitoring, however this hypercoagulability does not increase with prolonged treatment

Disclosure of Interest: None Declared.

Coagulation factor V, X, II, XIII – III

PO181-WED

Identification of two novel missense mutations causing severe factor XIII deficiency

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Background: Coagulation factor XIII (FXIII) is a transglutaminase with multiple roles in coagulation, wound healing, inflammation, maintenance of pregnancy, and other processes. Activated FXIII cross-links fibrin chains and covalently incorporates α_2 -antiplasmin

into the fibrin clot, which improves the mechanical stability of the clot and prevents its premature lysis. FXIII deficiency presents with bleeding, impaired wound healing and a higher incidence of abortions, and most cases result from a mutation in the gene encoding the catalytic A subunit of FXIII.

Aims: This study follows our recently published analysis of FXIII deficient patients from Pakistan and our aim was to detect and characterise novel causative mutations.

Methods: In patients suspected of FXIII deficiency, FXIII-A subunit antigen was measured by an ELISA, and screening for mutations in the *F13A* gene in FXIII-deficient patients was performed.

Results: We identified two novel missense mutations in the *F13A* gene, c.732C>A, p.Asp243Glu, and c.749T>C, p.Leu249Pro. Both are localised in exon 6 and the affected amino acids reside close to each other in the catalytic domain. Patients homozygous for either of these mutations have no detectable FXIII-A antigen in plasma and present with severe bleeding symptoms, while the heterozygous family members are asymptomatic.

Conclusion: Asp243 and Leu249 are both in contact with the activation peptide and they reside in close vicinity to the three previously described mutated residues (Ser19Pro, Met242Thr, Arg252Ile). This suggests that correct positioning of the activation peptide is important for the proper folding of the nascent FXIII-A subunits.

Disclosure of Interest: None Declared.

PO182-WED

First grounding for complete structural characterization of FXIII heterotetramer: successful disulfides!

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Background: The plasma circulating FXIII heterotetramer complex is composed of non covalently linked homodimers of FXIIIA₂ and FXIIIB₂ subunits. In order to unveil the inexplicable structure of FXIIIA₂B₂ heterotetramer, the initial prerequisite is the large-scale expression of both subunits. While the FXIIIA subunit has successfully been produced in large scale in yeast-based expression systems, the presence of high number of disulfide bonds makes it difficult to express FXIIIB in large scale.

Aims: To express and purify the rFXIIIB (recombinant FXIIIB) and its individual sushi domains in order to characterize the intra/inter domain interactions within the FXIII heterotetramer.

Methods: The *E. Coli* S30 based cell free expression system was used to express and purify full length FXIIIB subunit and 10 individual sushi domains. To aid the correct formation of disulfide bonds in FXIIIB (and sushi domains), reactions were set under non-reducing conditions in the presence of varied mixture of oxidising and reducing Glutathione buffers, complexed with PDI (protein disulfide isomerases) and later with in house produced bacterial disulfide isomerases DsbA & DsbC (expressed and purified in *E. Coli*).

Results: Using *E. Coli* S30 based cell free expression system, we were able to successfully clone, express and purify rFXIIIB and rSushi domains (1-10). The yield of protein thus obtained from 1 mL of reaction mix was upto 10 mg, but only in the presence of DsbA & DsbC.

Conclusion: Disulfides play an important role in providing intrinsic stability to secreted plasma proteins, one of which being plasma FXIIIB subunit. With the successful cloning, expression and purification of rFXIIIB and rSushi domains we have shown that the cell-free based expression system is appropriate for the expression and purification of coagulation related/based proteins (like FXIIIB) with multiple disulfide bonds. Openness of Cell-free systems allows flexibility and henceforth, the standardisations were relatively easy and fruitful.

Disclosure of Interest: None Declared.

PO183-WED

Revisiting the mechanism of coagulation factor XIII activation in plasma

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Background: The plasma circulating zymogenic protransglutaminase FXIII is a heterotetramer, composed of homodimers FXIIIA₂ and FXIIIB₂. It is physiologically activated by the combined action of Calcium and Thrombin. In light of certain new results we have revisited the concerted model of action in which conformational changes and proteolytic cleavage, combine to dissociate FXIIIB₂ subunit and expose the catalytic triad of FXIIIA₂.

Aims: To gain a deeper insight into the concerted model of action for the physiological activation of FXIII.

Methods: Two main sources of FXIIIA₂ were used: a) recombinant FXIIIA₂ expressed & purified using *Yeast expression* system b) FXIII heterotetramer isolated from *Fibrogammin P* (CSL Behring). The rFXIIIB₂ molecule (Zedira) was used for spiking the assays. The activation profile was determined physiologically using the FXIIIA generation assay, and non-physiologically by biochemical separation methods (Gel filtration, SDS PAGE, Mass spectroscopy).

Results: A primary observation in this study was that spiking rFXIIIB₂ into FXIIIB₂ deficient conditions (non-physiological), increased the rate of activation of FXIIIA₂. In lieu of this study, we repeated certain classical FXIIIA activation assays with our set of conditions and observed that although Thrombin is needed for proteolytic cleavage of activation peptide; low physiological Calcium concentrations play a definitive role in separation of the FXIIIB₂ subunits from the heterotetramer and enable the complete activation of the FXIIIA₂ subunit.

Conclusion: Changes in physiological/non-physiological concentrations of Calcium (1–25 mM) might lead to variability in FXIII activation rates. The activation rate enhancing role of the FXIIIB₂ subunit is most likely mediated by its interaction with factors such as Fibrinogen.

Disclosure of Interest: None Declared.

PO184-WED

Mutating core structural disulfides of the FXIII B subunit as a means to uncover the functional roles of individual FXIIIB subunit sushi domains

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Background: The FXIIIB₂ subunit is the protective component of the coagulation Factor XIII A₂B₂ heterotetramer. It is a filamentous dimer composed of ten repetitive but functionally distinct sushi domains. Limited information exists on the functional status of the FXIIIB subunit sushi domains.

Aims: To characterize induced core structural disulfide mutations in FXIIIB subunits in terms of their effect on stability, secretion and intra/inter chain protein interaction.

Methods: Mammalian expression vectors carrying the *F13B* cDNA insert were mutated individually for all core FXIIIB subunit structural disulfide bonds (n = 20). The mutated constructs along with the wild type were transfected into HEK293T cell lines. The expression products post 36 h of transfection were evaluated using various assays

including ELISA, western blot, Native PAGE's and confocal microscopy to determine their effect on secretion, stability and protein interaction. Molecular dynamic simulation was performed on disulfide mutated models of FXIIIB sushi domain to determine their putative structural impact.

Results: The mutated disulfide bonds showed variable degrees of influence on the stability (only seven out of the 20 mutated disulfide bonds showed significant extracellular antigenic presence), secretion (e.g. mutated *p.C91A* showed intracellular accumulation) and protein interaction of the expressed rFXIIIB₂ (recombinant) subunit (e.g. *p.C396A* showed weak dimer formation).

Conclusion: The mutated disulfide bonds and therefore their corresponding sushi domains can be segregated into the following functional groups: a) which have a major influence on secretion b) which influence dimerization c) which influence hetero-tetramerization i.e. interaction with the FXIIIA₂ subunit d) rate of biosynthesis of FXIIIB₂ subunit.

Disclosure of Interest: None Declared.

PO185-WED

The activation peptide of coagulation factor XIII is vital for its expression and stability

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Background: The activation peptide of human coagulation factor XIII (AP-FXIII) consists of the first 37 amino acids of the N-terminus and holds the FXIII in an inactive state. Thrombin induces FXIII activation by cleaving off the AP-FXIII which is then released into plasma.

Aims: The aim of this study was to investigate the role of AP-FXIII in the expression and stability of FXIII.

Methods: We cloned thirteen FXIII variants with progressive truncations of the activation peptide from the N-terminus (delN-FXIII-A), expressed them in mammalian cells and measured their thermostability, activation and transglutaminase activity. We further used in silico calculations to analyse the stability of hypothetical delN-FXIII dimers and to identify crucial motifs within AP-FXIII.

Results: Variants with deletions longer than the first ten amino acids were not expressed as proteins. In silico calculations indicated that the sequence ⁸FGGR¹²R plays a substantial role in intersubunit interactions in the FXIII-A₂ homodimer. In agreement with this prediction, the temperature stability of delN-FXIII variants decreased with increasing length of deletion, and delN variants del1-9, del1-10 had slightly increased activation rates. These results may suggest a role of the N-terminus of AP-FXIII in dimer stability. Substantial sequence homology was found among activation peptides of vertebrate and even invertebrate (crustacean) FXIII-A orthologs, which further supports our conclusion.

Conclusion: We conclude that deletion of eleven or more N-terminal amino acids disrupts intersubunit interactions, which may prevent FXIII-A₂ homodimer formation. Therefore, AP-FXIII plays an important role for stability of the FXIII-A₂ dimer.

Disclosure of Interest: None Declared.

PO186-WED

The B-subunit of coagulation factor XIII does not specifically interact with complement factors

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Background: The B-subunit of coagulation factor XIII (FXIII-B) has 10 Sushi domains (also termed SCR or CCP domains) and shows a high degree of sequence homology with complement factor H (fH) and C4b-binding protein (C4BP).

Aims: The aim of this study was to investigate whether FXIII-B specifically binds to complement C3b and C4b and whether it exerts cofactor activity in the degradation of C3b and C4b similar to fH and C4BP. **Methods:** Binding of FXIII-B to complement C3b and its degradation fragment iC3b was analysed by surface plasmon resonance (SPR). C3b was generated directly on a sensor chip via convertase-mediated deposition, and converted to iC3b by factor I (fI) and fH. FXIII-B and fH were injected to both surfaces. In order to test for cofactor activity in the fluid phase, we incubated C3b or C4b with fI in the presence of FXIII-B and/or fH or C4BP, respectively, for up to 20 min. The C3b and C4b degradation products were analysed by SDS-PAGE.

Results: In the SPR experiment we observed typical binding curves for fH at a concentration of 200 nM, whereas even high concentrations of 10 µM of FXIII-B only showed low binding to C3b. No significant binding was observed for either protein to iC3b. In the cofactor activity experiments, C3b and C4b cleavage products generated by fI in the presence of fH or C4BP could be clearly detected after 5 min of incubation. In the presence of FXIII-B, however, no C3b and C4b cleavage products were generated after 20 min of incubation. Furthermore, FXIII-B did not affect the cofactor activity of fH and C4BP.

Conclusion: Based on the structural similarity and high degree of sequence homology of FXIII-B with the complement regulators fH and C4BP, we hypothesised that FXIII-B might interact with complement proteins. However, in the present study we found neither any evidence for specific binding of FXIII-B to C3b or iC3b, nor for FXIII-B cofactor activity in the fI-dependent degradation of C3b and C4b.

Disclosure of Interest: None Declared.

PO187-WED

The effect of factor XIII B subunit polymorphisms on factor XIII levels and on the risk of coronary artery disease; interaction with fibrinogen level and factor XIII a subunit P.VAL34LEU polymorphism

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Background: The carrier B subunit of factor XIII (FXIII-B) is essential for keeping the catalytic A subunit (FXIII-A) in the circulation. FXIII-B is a polymorphic protein, p.His95Arg and intron K nt29756 C>G are its most common polymorphisms. The latter leads to a novel splice acceptor site and to the replacement of 10 C-terminal amino acids by an alternative sequence of 25 amino acids. Although the effect of FXIII-A polymorphism on the risk of coronary artery disease (CAD) has been intensively investigated, no such investigation has been carried out on FXIII-B polymorphism.

Aims: The aim of the case-control study was to explore the effect of FXIII B polymorphisms on the risk of CAD and on FXIII levels.

Methods: 687 patients admitted for coronary angiography to investigate suspected coronary artery disease and 994 individuals representing the Hungarian population were enrolled in the study. Patients were classified according to the extent of coronary atherosclerosis (CAS) and history of myocardial infarction (MI). The F13B gene was genotyped for p.His95Arg and for intron K nt29756 C>G polymorphisms and the F13A1 gene for p.Val34Leu polymorphism. FXIII activity and antigen levels were determined by ammonia release assay and ELISA, respectively.

Results: The p.His95Arg polymorphism did not influence the risk of CAS or MI. The FXIII-B intron K nt29756 G allele provided significant protection against CAS and MI in patients with a fibrinogen level in the upper tertile. However, this effect prevailed only in the presence of the FXIII-A Leu34 allele, and a synergism between the two polymorphisms was revealed. Carriers of the intron K nt29756 G allele had significantly lower FXIII levels, and FXIII levels in the lower tertile provided significant protection against MI. **Conclusion:** The combination of FXIII-A p.Val34Leu and FXIII-B intron K polymorphisms was protective against CAD at high fibrinogen level and their protective effect is related to decreased FXIII levels.

Disclosure of Interest: None Declared.

PO188-WED

FXIII-A V34L affects thrombus cross-linking, but not size, in a murine thrombosis model

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Background: Factor XIII-A V34L is a natural variant with a substitution close to the thrombin cleavage site, which undergoes more rapid thrombin activation, yet paradoxically associates with a protective effect against myocardial infarction in clinical studies. The effects of FXIII-A V34L on thrombus formation *in-vivo* are still unknown.

Aims: To study the effects of recombinant human FXIII-A variants on thrombus formation *in-vivo*.

Methods: rhFXIII-A variants (WT, V29A, E30A, G33A, V34A, V34L, V34M, V35A) were produced in *E. coli*. The FXIII-A activation rate was assessed by cleavage of activation peptide by thrombin. Factor XIII activity was determined using a pentylamine-biotin incorporation assay. FXIII^{-/-} and parent (wild-type) mice were injected with Alexafluor488-fibrinogen and Alexafluor680-A15 peptide (α_2 -AP cross-linking site), $\pm$ rhFXIII-A. FeCl₃ was applied to the femoral vein, and clot formation was visualised using intra-vital microscopy.

Results: rhFXIII-A variants V29A, E30A, G33A, V34A and V35A showed decreased, whereas V34L and V34M showed increased, activation rates. Similar changes were observed for FXIII activity, except E30A and V35A (unchanged). The rhFXIII-A variants with the highest (V34L) and lowest (G33A) activity, as well as WT, were used *in-vivo*. The V34L variant resulted in increased A15 incorporation compared to parent and FXIII^{-/-} WT mice, whereas the G33A variant had similar incorporation rate as that of the FXIII^{-/-} mice. Thrombus growth was reduced in FXIII^{-/-} and G33A mice compared with WT and parent mice, whereas V34L mice showed similar thrombus growth as parent and WT mice.

Conclusion: This study shows that a naturally occurring FXIII-A variant with high thrombin activation rates (V34L) results in enhanced thrombus stabilisation and normal thrombus growth *in-vivo*. These data indicate that FXIII-A V34L may be prothrombotic in this model and its reported cardioprotective effect is therefore unlikely explained by reduced thrombosis.

Disclosure of Interest: None Declared.

PO189-WED

Platelet factor XIII-A translocates from the activated platelet surface onto adjacent fibrin fibres

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Background: We have previously shown that cytoplasmic FXIII-A is exposed on the stimulated platelet surface and mediates extracellular cross-linking reactions. However, the processes involved in externalization and its association with the fibrin network are not defined.

Aims: To determine the mechanisms involved in exposure of FXIII-A on stimulated platelets and study its translocation into the fibrin network.

Methods: Washed platelets were prepared from whole blood by centrifugation. Clots were generated from FXIII-depleted plasma $\pm$ platelets. In some cases a transglutaminase (TG) inhibitor; theophylline and prostaglandin E1 (PGE₁); ARG-GLY-ASP-SER (RGDS); cytochalasin D or tirofiban were included.

Results: Inclusion of platelets in FXIII-depleted plasma clots revealed movement of FXIII-A from the membrane onto extending fibrin fibres. In contrast a homogenous distribution was observed with exogenous FXIII and in the absence of platelets no FXIII staining was detected. Pre-incubation of platelets prior to stimulation with PGE₁ and theophylline, to prevent aggregation and degranulation, abolished exposure of FXIII-A. Similarly, FXIII-A could not be detected on fibrin fibres in clots and treated platelets were unable to stabilize FXIII-depleted thrombi. Inclusion of a TG inhibitor showed that FXIII-A activity was required for movement from the platelet membrane onto fibrin fibres. Blocking the integrin $\alpha_{IIb}\beta_3$, which binds fibrin, with tirofiban or RGDS inhibits movement of FXIII-A to fibrin. This suggests that movement of FXIII-A from the membrane requires platelet-bound fibrin and is mediated via $\alpha_{IIb}\beta_3$. Cytochalasin D treatment, which inhibits actin polymerization, reduced transfer of FXIII-A into the fibrin network, suggesting that reorganisation of the cytoskeleton upon platelet activation may facilitate this process.

Conclusion: Activated platelets expose functional FXIII-A on their membrane which translocates onto bound fibrin via an $\alpha_{IIb}\beta_3$ -dependent mechanism that requires TG activity.

Disclosure of Interest: None Declared.

PO190-WED

Resident tissue macrophages are the probable source of plasma clotting factor XIII-A

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Background: FXIII-A is present in plasma and abundant within platelets. Although early studies proposed a platelet origin for plasma (p) FXIII-A, we have observed that pFXIII-A is unaltered in Mpl^{-/-} (thrombopoietin receptor), knockout mice, which are both thrombocytopenic and deficient in platelet precursors. FXIII-A is also constitutively expressed in a subpopulation of cells in heart, aorta and brain, identified as resident tissue macrophages, (RM). Certain populations of RM are maintained independently of the adult bone marrow/monocyte (BM) lineage.

Aims: To identify the cell-type that secretes pFXIII-A.

Methods: Transgenes expressing Pf4-cre (thrombopoietic deletion) or CD11b-cre (monocytic deletion) and LysM-cre knock-in alleles (myeloid deletion) were introduced into mice floxed in exon 7 of the *F13A* gene. Plasma and platelet FXIII-A transglutaminase activity measure-

ments were corroborated by FXIII-A immunoblotting. FXIII-A mRNA was assayed by RT-PCR. Fractional genomic (g) deletion was assayed by qPCR of gDNA, and calibrated with known mixtures of gDNA from floxed and global knockout mice.

Results: Pf4F-cre.FXIII-A^{fllox/flox} mice fully depleted platelet FXIII-A, while plasma FXIII-A was reduced by 81%. Further reduction was not achieved by introducing two Pf4-cre alleles. Partial reductions in pFXIII-A were obtained with CD11b-cre and LysM-cre, without corresponding falls in platelet FXIII-A. Pf4-cre caused limited (1-2%) recombination in gDNA of various organs but extensively depleted FXIII-A mRNA in heart, brain and artery, while organ FXIII-A mRNA was preserved in Mpl^{-/-} mice, confirming that the expressing cells are not BM-derived thrombopoietic cells. Residual aortic mRNA (mRNA) after -cre deletion most closely mirrored residual pFXIII-A (Enz): Pf4-cre, mRNA = 10%, Enz = 19%; CD11b-cre, mRNA = 62%, Enz = 62%; Pf4-cre + CD11b-cre, mRNA = 6%, Enz = 9%; LysM-cre, mRNA = 51%, Enz = 37%.

Conclusion: The cell-type that maintains plasma FXIII-A is similar to, and may correspond to, the arterial RM.

Disclosure of Interest: None Declared.

Coagulation factor VIII – Factor IX – III

PO191-WED

Effect of stable GABARAP knockout on the FVIII secretion

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Background: Few proteins are known to be involved in the secretion of coagulation factor VIII. The two chaperon systems calnexin/calreticulin (CNX/CRT) and immunoglobulin binding protein (BiP) help in the correct folding of FVIII, while the heterodimeric cargo trafficking complex LMAN1/MCFD2 transports the protein in coating protein II (COPII)-coated vesicles to the Golgi. Using the yeast two-hybrid system we previously identified Gamma-aminobutyric acid receptor-associated protein (GABARAP) as a new potential interacting cargo protein for FVIII B domain.

Aims: Our goal was to further verify the putative interaction between GABARAP and FVIII and to perform functional assays to study the role of GABARAP in the secretion of FVIII.

Methods: We performed immunoprecipitation in COS mammalian cells and we generated HEK293-stable FVIII secreting cells that we used to knockout endogenous GABARAP by either transient siRNA or stable knockout using the CRISPR/Cas9 system.

Results: Both full-length (FVIII wt) and the B domain deleted (BDD) FVIII showed an interaction with GABARAP in COS cells. We could prove that a transient knockout of GABARAP by siRNA in both transiently FVIII-transfected and in stable FVIII-expressing HEK293 cells lead to a reduced FVIII secretion. Finally the generation of an endogenous GABARAP knockout using the CRISPR/Cas9 system in stable FVIII expressing cells clearly verified its involvement in the FVIII secretion, by showing abnormal levels of secreted FVIII.

Conclusion: We confirmed GABARAP as a protein involved in the secretion of FVIII molecule.

Disclosure of Interest: None Declared.

PO192-WED

Dimeric organization of membrane-bound factor VIII on lipid nanotubes

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Background: Factor VIII is an essential protein for blood hemostasis. Its active form FVIIIa is the co-factor to the serine protease Factor IXa (FIXa) within the intrinsic tenase (FVIIIa-FIXa) complex. Understanding the membrane-induced structural changes in FVIII upon assembly of the FVIIIa-FIXa complex are physiologically important and of clinical interest. Resolving the FVIII membrane-bound organization is a critical step towards understanding the structural basis of FVIII function and the FVIIIa-FIXa complex assembly.

Aims: The goal of the presented research is to resolve the functional FVIII and FVIIIa membrane-bound organization by cryo-electron microscopy (cryo-EM) and tomography, and generate a new structure-based model for the FVIIIa-FIXa complex assembly and function.

Methods: pFVIII and pFVIIIa were helically reorganized on single bilayer lipid nanotubes (LNT) at close to physiological conditions. Cryo-EM and structure determination was carried out by helical and single particle tomography (SPT) analyses. The FVIII structure was fitted within the membrane-bound FVIII dimer map to define the protein-protein and protein-membrane interactions.

Results: In this work we show by cryo-electron microscopy (cryo-EM) and electron tomography (ET) that FVIII organizes as dimers when bound to negatively charged lipid nanotubes (LNT) at close to physiological conditions. We have calculated a pFVIII-LNT helical reconstruction at 15.5 Å and a SPT reconstruction at 20.5 Å from electron tomograms of the same pFVIII-LNT helical tubes, confirming the dimeric membrane-bound organization of FVIII.

Conclusion: Structure determination by cryo-EM and electron tomography can unambiguously define the oligomeric state of membrane-bound FVIII at intermediate resolution (10–25 Å) by applying two complementary structural approaches, helical and SPT. The resolved membrane-bound FVIII dimeric organization is an important step towards understanding the molecular recognition mechanisms supporting the FVIIIa-FIXa complex assembly.

Disclosure of Interest: None Declared.

PO193-WED

Impact of inhibitors to epitopes of factor VIII on thrombin generation

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Background: Factor (F) VIII inhibitors develop as alloantibodies in 20-30% of severe Hemophilia A (HA) patients. Patients with high-titer inhibitors require treatment with bypassing agents [recombinant factor VIIa (rfVIIa) or activated prothrombin complex concentrate (aPCC)] to control bleeding, but have variable individual responses. We hypothesize that epitope specificity may help determine individually tailored therapy for HA patients with high titer inhibitors

Aims: Determine the impact of inhibition of select epitopes of FVIII on thrombin generation (TG) using the CAT assay

Methods: FVIII deficient plasma was spiked with recombinant FVIII (rFVIII). Five murine anti-FVIII non overlapping monoclonal antibodies (MAbs) were used. Two MAbs targeted A2 domain (I62, B161), two C2 domain (3G6, 3D12) and one A3 domain (F147) of FVIII. TG was performed at 37°C for 60 min using CAT (Thrombino-

scope BV, Maastricht, The Netherlands). IgG2a and IgG2b were isotype controls. Lag Time, Peak Thrombin, and Endogenous Thrombin Potential (ETP) were analyzed

Results: TG was obtained using 1U/ml, rFVIII in FVIII deficient plasma. Each anti-FVIII MAb was added to achieve $5 \mu\text{g mL}^{-1}$ final concentration. All experiments were performed using both 1pM tissue factor (TF) and 5pM TF triggering reagents. Results were reproducible with coefficient of variation of less than 10%. MAbs F147 (66% ETP inhibition) and 3D12 (65%) displayed the most significant inhibition on TG. MAb I62 showed 48% ETP inhibition, MAb B161 30%, and 3G6 24% as compared to respective control antibodies

Conclusion: We assessed the effect of five inhibitory non-overlapping MAbs on TG. We concluded that thrombin generation was inhibited in the following order of domain specificity A3 > C2 > A2.

We intend to determine in vitro effectiveness of rFVIIa and aPCC on TG in plasma with inhibitors to different epitopes on FVIII, which may improve our ability to individualize selection of one agent for clinical utility

Disclosure of Interest: None Declared.

PO194-WED

Molar specific activity of factor VIII concentrates

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Background: The specific activity is a quality parameter that can be used to monitor the consistency of the production of plasma-derived (pd) or recombinant FVIII concentrates. However, it does not allow to directly compare the activity of different FVIII molecules on a molar basis due to difficulties to exactly determine low FVIII protein concentrations or due to the fact that the exact molecular weight is not known as in case of pd FVIII or 'full-length' rFVIII.

Aims: Therefore, it was the aim of the present study to establish a method for determination of the molar specific activity of FVIII products to directly compare their activity on a molar basis.

Methods: The activity of different FVIII concentrates was determined using a chromogenic substrate (ChS) FVIII activity assay (Coamatic FVIII test kit). The molar concentrations of the FVIII products were determined by complete activation in the presence of thrombin and quantification of the A1 domains released using RP-HPLC on a C4 column. The RP-HPLC measurements were standardized using the thrombin digest of a well-defined rVIII-SingleChain concentrate.

Results: As a result of the present study, the molar quantities could be determined for three lots each of different recombinant FVIII products and for one lot of pd FVIII. Together with the ChS FVIII activity assay results the molar specific activities were calculated. For the rVIII-SingleChain lots the molar specific activity calculated was $1.91 \pm 0.07 \text{ IU pmol}^{-1}$, for the ReFacto AF lots it was $1.83 \pm 0.05 \text{ IU pmol}^{-1}$, for the NovoEight lots it was $1.77 \pm 0.08 \text{ IU pmol}^{-1}$, for the Advate lots it was $1.53 \pm 0.06 \text{ IU pmol}^{-1}$, and for the pd FVIII lot it was $1.75 \text{ IU pmol}^{-1}$.

Conclusion: In conclusion, a versatile method could be established that allows the determination of the molar specific activity of FVIII products. The method is independent on the exact molecular weight or the protein content of the FVIII products and should also be applicable to FVIII products modified outside the A1 domain.

Disclosure of Interest: C. Horn Employee of: CSL Behring GmbH, S. Steede Employee of: CSL Behring GmbH, S. Schmidbauer Employee of: CSL Behring GmbH, H. Metzner Employee of: CSL Behring GmbH.

PO195-WED

High-purity, plasma-derived, pasteurised factor VIII concentrate in the treatment of patients with haemophilia A: update of a long-term observational study

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Background: Comprehensive, large-scale, non-interventional studies are effective tools to collect data on products in the post marketing authorisation period.

Aims: This study assessed the long-term effectiveness, tolerability and safety of a high-purity plasma-derived factor VIII concentrate, Beriate[®] (CSL Behring GmbH, Marburg, Germany) in the treatment of patients with haemophilia A (HA).

Methods: Previously untreated and previously treated patients with HA at any age, who had received Beriate[®] were enrolled. Based on the proceeding at the centres, patients were routinely seen and documented every 3–12 months. Parameters documented comprised effectiveness-, safety-, pharmacoeconomic-, and pharmacokinetic data, relevant concomitant diseases, and relevant concomitant medication.

Results: Up to now, 87 patients were enrolled into this study [median duration: 46 months (0–138)] and data from 941 visits were available for this analysis. The median age of the study population was 20.0 years (range: 0.1–74.6). Seventy-two patients suffered from severe-, ten patients from moderate-, and four patients from mild HA. In one patient, the information on severity of HA was missing.

81% of the patients (56/69) received prophylaxis with at least one infusion per week. Median average number of bleeds per year was 2.7 (0–33) and a median of 1.0 (0–83) infusions per bleeding was administered. 74% of the patients (54/73) experienced no major bleeds and 12.3% (9/73) experienced no bleed at all. Effectiveness of Beriate[®] was judged to be excellent or good in 98.4% of all bleeding.

Two cases of inhibitor development were reported; one inhibitor was transient and the second inhibitor was treated with immune tolerance therapy. There was no proven virus transmission for hepatitis A-, hepatitis B-, hepatitis C- or human immunodeficiency virus.

Conclusion: The results included in this interim analysis confirm the very good efficacy, tolerability, and safety of Beriate[®].

Disclosure of Interest: R. Klamroth Grant/Research Support from: CSL Behring, C. Niekrens Grant/Research Support from: CSL Behring, S. Holzhauser Grant/Research Support from: CSL Behring, R. Fischer Grant/Research Support from: CSL Behring, C. Heller Grant/Research Support from: CSL Behring, P. Sommerer Employee of: CSL Behring GmbH, K. Kurnik Grant/Research Support from: CSL Behring.

PO196-WED

Expression, purification and characterization of codon optimized coagulation factor VIII

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Background: Therapeutic coagulation factor VIII (FVIII) products are used to treat FVIII deficiency (Hemophilia A). The expression and

purification of FVIII are challenging due to low levels of its expression. One approach to increase protein production is using codon optimization. Such approach was previously shown to increase FVIII activity in cell culture and *in vivo* (Ward et al, Blood 2011, 117:798-807).

Aims: To express and purify codon optimized FVIII (CO) at the laboratory scale and investigate its equivalence to wild-type FVIII (WT).

Methods: Expression cassettes for CO and WT (B-domain deleted (BDD) FVIII with C-terminal poly-His tag) were inserted into a lentivirus-based vector. CHO cells were repeatedly transduced with the vectors followed by isolation of clones stably secreting CO and WT. Both proteins were expressed in adherent cultures, purified using two steps of affinity chromatography, specific to poly-His tag and FVIII, and analyzed for the FVIII activity in a chromogenic assay and for the secondary structure by far-UV circular dichroism.

Results: For the expression, three different cell lines were tested, with CHO cells being the most suitable. For each of WT and CO, several clonal lines stably expressing the proteins were produced. The estimated levels of the secretion were 0.1–0.7 mg L⁻¹ (24 h)⁻¹ for WT and 0.4–1.2 mg L⁻¹ (24 h)⁻¹ for CO. For each FVIII variant, selected clonal lines demonstrated stable levels of the secretion for more than 20 days of cultivation in the same flasks. In SDS-PAGE, purified WT and CO produced the same band patterns, which corresponded to that of a commercial BDD FVIII. Both WT and CO were found to have similar FVIII specific activities and secondary structures.

Conclusion: Relatively high level of FVIII expression can be achieved using combination of codon optimization, multiple rounds of lentiviral transduction and clone selection. No structural or functional differences were found between the wild-type and codon optimized FVIII.

Disclosure of Interest: None Declared.

PO197-WED

Whole-exome sequencing approach to study inhibitor development in Italian severe hemophilia A patients

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Background: Hemophilia A (HA) is an inherited bleeding disorder that is predominantly characterized by deficiencies in Factor VIII (FVIII) due to mutations in the *F8* gene. The current treatment of patients suffering from HA consists of infused plasma-derived or recombinant FVIII products. However, approx. 30% of patients develop neutralizing antibodies (inhibitors) to the infused FVIII therapy. Previous genotyping studies have identified several polymorphisms associated with genes involved in immune responses such as HLA locus, IL-10, CTLA4 or TNFA as genetic risk factors underlying Inhibitor development.

Aims: To identify novel genetic elements underling the risk of inhibitor development in severe HA patients using next-generation DNA sequencing (NGS).

Methods: We performed whole-exome sequencing analysis of 26 Italian severe HA patients with inhibitor (N = 17) and without (N = 9) inhibitor. Exome sequence capture was performed using the SeqCap EZ design library (NimbleGen) and sequencing was conducted on an Illumina HiSeq 2000.

Results: Data analysis revealed 1,865,389 single nucleotide variants and 82,537 small insertions/deletions that passed the quality control with an average sequencing read-depth of 53. We identified 1360 novel gene targets statistically significant ($P \leq 0.05$) for Cochran-Armitage and Fisher's Exact statistical tests. Hierarchical clustering of these gene targets showed two distinct patterns of homozygous variants that could have protective or nocive role in inhibitor development. Further filtering analysis revealed 221 risk and 190 protective variants that occur in homozygosity. When looking solely at coding variants predicted to be damaging, 40 putative targets were identified.

Conclusion: The gene targets discovered in our analysis affect pathways implicated in auto-immunity and immune system, indicating that inhibitor development is a complex multifactorial disease. A replication study using TaqMan assays is underway to confirm the NGS results.

Disclosure of Interest: M. M. Gorski: None Declared, K. Blighe: None Declared, I. Garagiola: None Declared, S. Seregni: None Declared, M. E. Mancuso: None Declared, E. Santagostino: None Declared, L. A. Lotta: None Declared, F. Peyvandi Grant/Research Support from: Alexion, Bayer, Biotest, Kedrion Biopharma, Novo Nordisk, Consultant for: Grifols, Kedrion Biopharma, LFB, Octapharma, Speaker Bureau of: Alexion, Baxter, Bayer, Biotest, CSL Behring, Grifols, Novo Nordisk.

PO198-WED

Minigene approach to characterize a novel splice site mutation in human F8 gene causing severe haemophilia A

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Background: Haemophilia A (HA) is caused by a large number of molecular events in *F8*, and missense mutations are the most prevalent type.

Aims: Two new *F8* missense mutations (c.670G>A; p.G224R and c.3674A>G; p.Q1225R) were identified in a woman with reduced plasma level of FVIII activity (19%) and antigen (14%). The substitution c.670 G>A affected the last nucleotide of exon 5. The aim of this study was to determine the functional consequences of these molecular genetic defects on the factor VIII (FVIII) protein.

Methods: A combination of *in silico* tools (Alamut 2.6.0, *InteractiveBiosoftware*) and *in vitro* functional tests such as variant expression in COS-1 cells and minigene models in HeLa cells was used to determine the pathogenicity of these variations.

Results: *In silico* analysis predicted that the missense variant p.G224R could be pathogenic but FVIII activity and antigen levels for all mutants (p.G224R, p.Q1225R and p.G224R+ p.Q1225R) expressed into the conditioned media of COS-1 cells were similar to FVIII WT. Splice-site prediction tools predicted that the c.670G>A variation would affect the 5' splice site of *F8* exon 5. Sequencing of the RT-PCR products from the mutant minigene (c.670A) showed complete *F8* exon 5 skipping, whereas normal splicing occurred in the WT minigene (c.670G).

Conclusion: From our results, the missense variation p.Q1225R has no functional impact. The mutation c.670C>A (p.G224R) is a splice site mutation, responsible for *F8* exon 5 skipping, creating a frame shift. In conclusion, this woman is probably carrier of severe HA and skewed X chromosome inactivation may explain her low plasma FVIII activity.

Disclosure of Interest: None Declared.

PO199-WED

Clearance receptor interaction and half-life prolongation of pegylated recombinant full-length factor VIII in mice

Schrenk G, Schiviz A, Knappe S, Bauer A, Gritsch H, Billwein M, Michaela S, Turecek P, Dockal M, Putz M, Hoellriegel W and Scheifflinger F

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Background: Baxter and Nektar have developed a longer acting recombinant (r) factor (F) VIII (BAX 855), a full-length rFVIII coupled to

polyethylene glycol (PEG). The low-density lipoprotein-receptor-related protein-1 (LRP) is considered to be a determinant of FVIII clearance.

Aims: To compare the interaction of BAX 855 and unmodified rFVIII with LRP *in vitro* and the pharmacokinetics (PK) in mice lacking both endogenous FVIII and von Willebrand factor (VWF).

Methods: Binding kinetics were determined by surface plasmon resonance technology, where LRP was immobilized on a sensor chip and BAX 855/rFVIII used as analytes. An ELISA combined with a chromogenic assay for FVIII was used to measure the binding capacity and inhibitory effect of VWF. PK properties of rFVIII and BAX 855 were evaluated in a VWFx FVIII double knockout (dko) mouse model. After i.v. administration of 200 IU kg⁻¹ rFVIII, FVIII activity plasma levels were determined at several time points.

Results: For unmodified rFVIII, mean equilibrium dissociation constant (K_D) values were 58 and 119 nM for BAX 855, indicating a marginally decreased affinity for BAX 855. Importantly, the binding capacity for BAX 855 was 50% less than that for rFVIII. VWF interfered with FVIII binding to LRP similarly for BAX 855 and rFVIII. In VWFx FVIII dko mice, unmodified rFVIII was rapidly cleared from the circulation and was undetectable 3 h after injection. The estimated half-life was 0.3 h. In contrast, terminal half-life of BAX 855 was prolonged to 4.3 h, suggesting reduced clearance of BAX 855 in the absence of the chaperone VWF.

Conclusion: *In vitro* results show that PEGylation of rFVIII reduces its interaction with the LRP clearance receptor and substantially protects rFVIII from *in vivo* clearance, which is an important determinant for the increased circulation time of PEGylated rFVIII. This finding provides a solid molecular basis for the prolonged half-life compared with unmodified rFVIII observed in FVIIIxVWF dko mice.

Disclosure of Interest: G. Schrenk Employee of: Baxter Innovations GmbH, A. Schiviz Employee of: Baxter Innovations GmbH, S. Knappe Employee of: Baxter Innovations GmbH, A. Bauer Employee of: Baxter Innovations GmbH, H. Gritsch Employee of: Baxter Innovations GmbH, M. Billwein Employee of: Baxter Innovations GmbH, S. Michaela Employee of: Baxter Innovations GmbH, P. Turecek Employee of: Baxter Innovations GmbH, M. Dockal Employee of: Baxter Innovations GmbH, M. Putz Employee of: Baxter Innovations GmbH, W. Hoellriegel Employee of: Baxter Innovations GmbH, F. Scheiflinger Employee of: Baxter Innovations GmbH.

PO200-WED

Safety of PEG, PEG conjugates and pegylated recombinant human full-length coagulation factor VIII (BAX 855)

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Background: BAX 855 is a PEGylated human full-length recombinant coagulation factor VIII (rFVIII) based on Baxter's licensed rFVIII. The coagulation factor was modified with a PEGylation technology optimized to retain functionality of the FVIII molecule, improve its pharmacokinetic properties, and allow less frequent injections while maintaining efficacy.

Aims: To confirm that the excellent safety profile of Baxter's rFVIII remains unchanged after PEGylation.

Methods: Non-clinical safety studies with BAX 855 and its respective unbound PEG (PEG2ru20KCOOH) were conducted in several species. The distribution of a single dose of radiolabeled BAX 855 was further investigated in rats. Furthermore, available safety data on PEG alone and PEGylated biomolecules were reviewed and summarized for specific safety findings attributable to PEG or PEGylated biopharmaceuticals.

Results: Safety pharmacology studies in rabbits and macaques and repeated dose toxicity studies in rats and macaques identified no safety issues. Results of a distribution study in rats administered radiolabeled BAX 855 showed that radioactivity was completely excreted via urine as the major elimination route. A 28-day study in rats dosed with the unbound PEG constituent and major metabolite (PEG2ru20KCOOH) of BAX 855 showed no adverse and non-adverse effects.

Safety data for PEG and PEG-protein conjugates do not indicate any safety concerns associated with PEG at dose levels clinically relevant for BAX 855 therapy. Although vacuolation of certain cell types has been reported in mammals, no such vacuolation was observed with BAX 855 or with the unbound PEG constituent.

Conclusion: Non-clinical safety evaluation of PEG and BAX 855 as well as a review of available safety data on PEGylated biopharmaceuticals identified no safety signals, and the compound was successfully transferred into clinical development in patients with hemophilia A.

Disclosure of Interest: R. Stidl Employee of: Baxter Innovations GmbH, S. Fuchs Employee of: Baxter Innovations GmbH, M. Bossard Employee of: Nektar Therapeutics, J. Siekmann Employee of: Baxter Innovations GmbH, M. Putz Employee of: Baxter Innovations GmbH, P. Turecek Employee of: Baxter Innovations GmbH.

PO201-WED

Functional characterization of different factor VIII molecules with focus on phospholipid and platelet binding

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Background: Hemophilia A is caused by mutations in the factor VIII (FVIII) gene. Current treatment for hemophilia is FVIII replacement therapy, which, however, is limited by a short FVIII half-life, requiring frequent infusions. Thus, recombinant FVIII (rFVIII) molecules with an extended half-life, such as PEGylated rFVIII or rFVIII fused to an immunoglobulin Fc domain, have been developed.

Aims: To investigate functional aspects of different rFVIII molecules.

Methods: rFVIII¹ was cloned and expressed in HEK293H cells and purified by affinity and cation exchange chromatography. The other FVIII molecules were ready formulated. We analyzed unmodified full-length (fl)-rFVIII, PEGylated fl-rFVIII, genetically engineered B-domain deleted (BDD)-rFVIII, and rFVIII¹ for interaction with VWF and PLs using Biacore. Hemostatic activity in hemophilia patient plasma was assessed by calibrated automated thrombography (CAT). Thrombin generation was also analyzed using various phospholipid (PL) compositions and platelets. Discrepancies between FVIII chromogenic and 1-stage clotting assays were determined.

Results: Purification of rFVIII¹ yielded a pure and homogenous protein. The tested rFVIII proteins had similar hemostatic activity in standard activity assays and in VWF binding affinities. However, FVIII¹ showed lower thrombin generation than the other FVIII¹ at low PL or low platelet counts, indicating reduced PL and platelet binding of FVIII¹ at limiting surface conditions. Variability in activity assessment by 1-stage assays was lowest for fl-rFVIII.

Conclusion: Our data suggest that engineering of the FVIII molecule influences PL and platelet binding in plasma. Further studies are needed to better understand mechanisms of action and the role of distinct rFVIII domains of engineered FVIII proteins in support of extended half-life therapies for hemophilia.

Disclosure of Interest: S. Till Employee of: Baxter Innovations GmbH, S. Tippl Employee of: Baxter Innovations GmbH, M. Palige Employee of: Baxter Innovations GmbH, E. Boehm Employee of: Baxter Innovations GmbH, G. Schrenk Employee of: Baxter Innovations GmbH, J. Koehn Employee of: Baxter Innovations GmbH, H. Gritsch Employee of: Baxter Innovations GmbH, S. Knappe Employee of:

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PO202-WED

Molecular signature of LSECs: a F8 secreting endothelial cells

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Background: Historically, the cellular origin of the plasma F8 was controversial. Although the liver was accepted to be the main production site, it was still debated if the F8 originated from parenchymal or non-parenchymal cells. Recently, it was established, in both human and mice, that the sinusoidal endothelial cells of the liver (LSECs) and not the hepatocytes are the main F8 secreting cells. Additionally, other endothelial cells throughout the body contribute to plasma F8 levels but to lower extent.

Aims: In order to understand the cellular mechanisms responsible for the specificity of the F8 production and secretion to endothelial cells and more specifically to the LSECs, we have generated genome wide gene expression and DNA methylation profiling from fetal human hepatic sinusoidal endothelial cells, hepatic cells and several other endothelial cells.

Methods: We used the Illumina microarray platform for expression and methylation profiling and several bioinformatics methods were used to analyze the data. Values of selected loci were further verified using real time PCR and bisulfite pyrosequencing.

Results: Our results show a clear clustering (at FDR of <5%) of three distinguishable groups: 1) Hepatocytes, 2) LSECs and 3) rest of endothelial cells. Gene ontology analysis showed enrichment in GO-terms like regulation of cellular metabolic process for LSECs vs. endothelial cells and blood vessel development, cell adhesion and regulation of cell migration for LSECs vs. hepatocytes. When comparing LSECs to hepatocytes, we found that overexpressed genes in LSECs include TEK (TIE2), VWF, F2R and ADAMT15, while overexpressed genes in hepatocytes include FGA, FGB, Albumin and TF. Moreover, when LSECs and endothelial cells were compared to each other's, we found three fold more loci overexpressed in LSECs.

Conclusion: Our data reveals specific molecular signature that identify the F8 producing and secreting cells and highlight specific cellular pathways associated with this process.

Disclosure of Interest: None Declared.

PO203-WED

Ancestral reconstruction of coagulation factor VIII

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Background: The study of orthologous factor VIII (FVIII) molecules has led to several discoveries culminating in the approval of a recombinant porcine FVIII therapeutic. Several laboratories, including our own, have continued to exploit interspecies differentials in FVIII to design improved gene and protein-based therapeutics for hemophilia A.

Aims: To study the molecular evolution of interspecies differentials in FVIII through the reconstruction of inferred ancestral FVIII molecules.

Methods: A phylogenetic tree was constructed based on known FVIII sequences and the respective sequences at each ancestral node (An) were inferred. Eight recombinant An-FVIII molecules were selected for production from both human and rodent cell lines.

Results: All eight An-FVIII sequences encode secreted proteins that possess activity in human FVIII-deficient plasma. Transient and stable cell line expression comparisons revealed that a shared common ancestor (An-FVIII 53, ~90 Ma) of primates and rodents possesses a 9-fold higher expression rate than human FVIII. However, the greatest expression rate was observed for a more recent rodent ancestor (An-FVIII 68, ~30 Ma) which displays 14–20 fold greater expression than human FVIII. Following activation (a) by thrombin, FVIIIa loses its activity through spontaneous subunit dissociation. The shortest half-lives ($t_{1/2}$ = 1.5–2.4 min) were observed for An-FVIIIa along the human/primate lineage (An-FVIIIa 52, 53, 54, 57 and human), while the longest ($t_{1/2}$ = 14–16 min) were observed for more recent and current rodent FVIIIa (An 68 and mouse, respectively).

Conclusion: These data suggest that biosynthetic efficiency has decreased during primate evolution while the FVIIIa half-life has remained unchanged. Conversely in recent rodents, the half-life has become prolonged. Ancestral reconstruction of inferred FVIII sequences enables the rapid identification of functional sequences that can be engineered into future gene and protein therapeutics.

Disclosure of Interest: None Declared.

PO204-WED

A synonymous mutation in the F8 gene, C.120C>A; P.(L40 =), may cause mild hemophilia A

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Background: Missense mutations in the coagulation factor VIII gene (F8) are the etiology of most cases of mild hemophilia A.

Aims: We aimed to clarify the causative mutations of a particular patient with mild hemophilia A.

Methods: He had no known family history of hemophilia. Genomic DNA was extracted from peripheral blood cells. The entire F8 was amplified in 14 overlapping regions by long-PCR. The PCR products were equimolarly mixed and the nucleotide sequence was analyzed by next generation sequencing (NGS) using a MiSeq sequencing system (Illumina) with a Nextera XT DNA Sample Prep Kit (Illumina). Multiplex ligation-dependent probe amplification (MLPA) was carried out using an F8-P178 kit (MRC-Holland).

Results: The patient's plasma factor VIII (FVIII) activity and antigen levels were 20.6% and 17.5%, respectively. The patient was negative for anti-FVIII antibody. Type2N VWD was excluded by factor VIII binding assay. MLPA analysis revealed no duplications or deletions in the F8. As a result of NGS, two synonymous mutations, c.120C>A; p.(L40 =) and c.3864A>C; p.(S1288 =) were detected with high reliability. No other abnormalities that are thought to be causative mutations were detected in the F8. This result indicates the possibility that these two synonymous mutations are candidates for causative mutations. However, given that c.3864A>C; p.(S1288 =) is registered in dbSNP as rs1800292, where it is described as a 'benign' allele, it is unlikely to be causative. This suggests that the novel synonymous mutation, c.120C>A; p.(L40 =), may have been the cause of the patient's mild hemophilia A. *In silico* analyses of the synonymous codon substitution (CTC to CTA) of the L40 mutation indicated the creation of a new splicing site. Furthermore, it was also speculated that the significant decrease of the codon usage from the CTC (19.6/1000 codons) to the CTA (7.2/1000 codons) might impair protein synthesis.

Conclusion: The c.120C>A; p.(L40 =) mutation is not silent and may have been the cause of the patient's mild hemophilia A.

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PO205-WED

Long-term phenotypic correction of hemophilia A mice by non-viral *piggyBac* transposon vector

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Background: Gene therapy for hemophilia A is an attractive strategy for the treatment of the disease because continuous expression of coagulation factor VIII (FVIII) levels as low as 1-5% of normal have been shown to substantially ameliorate the bleeding phenotype in preclinical and clinical settings. Although several attempts have been made, systemic delivery of viral vectors into liver, however, resulted in a transient and low-level expression of FVIII because the large size of FVIII cDNA prohibited the efficient delivery. Moreover, there remain concerns over the safety of these approaches such as adverse immunological reactions or virus-mediated cytotoxicity.

Aims: To address this issue, we established the non-viral *piggyBac* transposon vector system, which can transfer a large transgene.

Methods: The full-length human FVIII cDNA was cloned into the *piggyBac* vector and the efficacy of new vector system was tested *in vitro* and *in vivo*.

Results: We tested the efficiency of the new vector system *in vitro* and confirmed the FVIII expression in culture media by the activity-sensitive coagulation assay. Hydrodynamic injection of these *piggyBac* vectors into hemophilia A mice via tail vein resulted in stable circulating level of FVIII in all the treated mice for over 300 days without developing anti-FVIII antibodies.

Conclusion: Our engineered vectors can facilitate the long-term expression of therapeutic transgenes *in vitro* and *in vivo*. This novel gene transfer strategy can provide the safe and efficacious delivery of full-length FVIII gene and merits further assessment.

Disclosure of Interest: None Declared.

PO206-WED

Discordant *in vitro* and *in vivo* activity of factor VIII mutants

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Background: Activity of Factor VIII (FVIII) mutants is often determined in 1-stage coagulation assays. However, their *in vivo* procoagulant potential remains unclear.

Aims: To compare the *in vivo* procoagulant potential of FVIII mutants p.R372A (thrombin activation), p.R1719T/p.R1721T (no light chain cleavage by FIXa or FXa) & p.I566R (defective FIXa binding) to 1-stage activity assays.

Methods: B-domainless FVIII variants were expressed in F8-deficient mice via hydrodynamic gene-transfer (HGT) and their hemostatic activity was assessed in a tail-clip bleeding assay. Blood loss was considered to be corrected when ≤ 0.15 mL (responder). Antigen (Ag) and 1-stage activity (Act) were determined.

Results: HGT of wt-FVIII resulted in a plasmid dose-dependent expression ($3\text{--}500$ U dL⁻¹) with an Act:Ag ratio of 0.98 ± 0.04 ($n = 75$). Increasing FVIII activity correlated with a gradually increasing % of responders: 30% for <20 U dL⁻¹ ($n = 10$), 82% for $20\text{--}50$ U dL⁻¹ ($n = 11$), 88% for $50\text{--}100$ U dL⁻¹ ($n = 8$) and 96% for >100 U dL⁻¹ ($n = 24$), compared to 10% ($n = 20$) responders in non-treated F8-deficient mice. All three mutants displayed discordant *in vivo* procoagulant activity compared to 1-stage activity. For mutant p.R372A (Act:Ag ratio 0.11 ± 0.02 ; no detectable chromogenic activity; $n = 34$), 14% ($n = 14$) of mice with an 1-stage activity ≥ 20 U/dl were responder, 5–6 fold lower than for wt-FVIII. For mutant p.R1719T/p.R1721T (Act:Ag ratio 1.2 ± 0.1 ; $n = 32$) a similar % of responders was found if activity was >50 U dL⁻¹. At activity levels between 20 and 50 U dL⁻¹, however, the % of responders (50%; $n = 10$) was lower than for wt-FVIII. Finally, mutant p.I566R (Act:Ag ratio 0.05 ± 0.03 ; $n = 11$), only two of seven mice (29%) with 1-stage activity > 20 U dL⁻¹ (max activity 59 U dL⁻¹) were responder, 2–3 fold lower than for wt-FVIII.

Conclusion: HGT provides an efficient tool to assess the procoagulant potential of FVIII mutants *in vivo*. Our data indicate that the 1-stage activity assay is a poor predictor of mutant FVIII activity *in vivo*.

Disclosure of Interest: A. Bazaa: None Declared, V. Muczynski: None Declared, C. Ioubière: None Declared, A. Harel: None Declared, T. Knudsen Employee of: Novo Nordisk, Måløv, Denmark, O. D. Christophe: None Declared, C. V. Denis: None Declared, P. J. Lenting Grant/Research Support from: Novo Nordisk.

PO207-WED

Beneficial effect of VWF in FVIII recovery of different FVIII concentrates in a mouse model of severe hemophilia with high inhibitor titers

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Background: We have previously reported the benefit of VWF-containing FVIII concentrates in restoring FVIII circulating levels in FVIII^{null} mice with low level of inhibitors (<5 BU). In clinical practice, inhibitor titers classify patients into low and high responder, helping to adapt treatment procedures.

Aims: To evaluate in a mouse model of severe hemophilia the differences of FVIII *in vivo* recovery between different FVIII concentrates – containing or not VWF – in the presence of high inhibitor levels (>5 BU).

Methods: Inhibitor IgG was purified (Protein G Sepharose) from a pool of human hemophilic plasmas. FVIII^{null} E16 Knockout (KO) mice ($N = 5\text{--}6$) previously infused with inhibitor to achieve low (<5 BU) or high (>5 BU) titers (or buffer as control), were administered FVIII therapeutic concentrates (100 IU kg⁻¹) from different sources: native pdFVIII/VWF complex, isolated pdFVIII and rFVIII. In addition, some animals were treated with *in vitro* preformed complexes of pdVWF plus FVIII (rFVIII+pdVWF and pdFVIII+pdVWF). After 5 min, plasma samples were obtained and FVIII:C was measured (chromogenic assay).

Results: FVIII recovery in control mice was similar for all FVIII concentrates (range: 108%–124%). In the presence of high titer inhibitors (6.1 ± 1.6 BU) FVIII recovery was higher for native pdFVIII/VWF when compared to concentrates of isolated FVIII (20.8% vs. 5.4% for

rFVIII and 1.2% for pdFVIII). That was the same behavior observed in low titer inhibitors (3.2 ± 0.4 BU): 71.1% vs. 31.4% for rFVIII and 25% for pdFVIII. In isolated FVIII concentrates premixed with pdVWF prior to infusion, FVIII recovery was only partially restored (rFVIII+pdVWF: 9.3% and 67.1%, pdFVIII+pdVWF: 9.7% and 45.2%, for high and low titer, respectively).

Conclusion: VWF-containing FVIII concentrates were more efficient in the restoration of FVIII circulating levels in *FVIII^{mut} E16 KO* mice even in the presence of high inhibitor titer. This effect was higher in native pdFVIII/VWF complex than in the complex of FVIII+VWF formed from the isolated proteins.

Disclosure of Interest: M. I. Bravo Employee of: Grifols, M. Costa Employee of: Grifols, S. Grancha Employee of: Grifols, J. I. Jorquera Employee of: Grifols.

PO208-WED

Variation in factor VIII measurement and classification of haemophilia A severity in the ECAT external quality assessment programme

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Background: Haemophilia A is an X-linked bleeding disorder caused by a deficiency of Factor VIII (FVIII). The plasma level of FVIII determines the classification and consequently the severity of haemophilia A. Treatment is based on FVIII plasma levels and bleeding phenotype. As a result, misclassification may lead to inadequate or delayed treatment. Hence, the ECAT external quality assessment programme aims to improve the results of clinical laboratories working in haemostasis and thrombosis.

Aims: To establish the variation in measured FVIII levels and classification of haemophilia A in the ECAT programme.

Methods: For this evaluation, six samples were selected with FVIII < 0.15 IU mL⁻¹ that were measured by 168–211 laboratories. The mean of all measured FVIII levels was defined as the apparently true value and was used to classify samples as mild (> 0.05 and < 0.40 IU mL⁻¹) moderate (0.01 – 0.05 IU mL⁻¹) or severe (< 0.01 IU mL⁻¹) haemophilia A according to the SSC/ISTH criteria. FVIII variation was compared between one-stage and chromogenic assays.

Results: In a sample with a true value of 0.009 IU mL⁻¹, 129 (64.2%) laboratories classified this sample correctly as severe haemophilia A. Classification was also correct in 204 (96.7%) laboratories in a sample with a true value of 0.10 IU mL⁻¹. FVIII levels around haemophilia cut-off values showed variability in severity classification, for example, a FVIII sample with a true value of 0.01 IU mL⁻¹ varied from 0.00 to 0.1 IU mL⁻¹. The range for levels with a true value of 0.05 IU mL⁻¹ was 0.00 – 0.25 IU mL⁻¹, also causing classification problems. The type of assay (one-stage vs. chromogenic) did not explain the variation in FVIII levels ($P > 0.05$).

Conclusion: Classifying haemophilia A patients based on FVIII level may be inaccurate when FVIII levels are close to the cut-off levels of haemophilia criteria. Since FVIII measurements are important for diagnosis and therapeutic management, research is needed to further improve FVIII testing.

Disclosure of Interest: None Declared.

PO209-WED

Pro-hemostatic activity of TFPI inhibition in hemophilia is well regulated by tissue factor and factor VIII

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Background: Tissue factor (TF), the cofactor for factor VIIa-catalyzed activation of factors IX and X, is an important initiator of hemostasis. Prominent TF expression has been described for vital organs (Drake, 1989). The main regulator of this extrinsic initiation pathway is tissue factor pathway inhibitor (TFPI). Low expression levels of TFPI were found in the brain, heart, and kidneys (Bajaj, 1999). Higher TFPI concentrations are present in synovial fluids of hemophilia A patients than in controls (Dargaud, 2012). These findings may explain why joints are the main bleeding site in patients with hemophilia.

Aims: To assess the regulation of coagulation by TFPI at increasing TF concentrations and hemophilic conditions.

Methods: As a model for hemophilia, blood and plasma were pre-incubated with goat anti human FVIII antibody to block FVIII activity. Thrombin generation in plasma from 13 individuals and in two plasma pools was triggered with 1 – 20 pM TF. Thromboelastometry (ROTEM) in FVIII-inhibited citrated blood from a healthy donor was initiated by adding 0.044 , 0.2 , or 1 pM TF. TFPI activity was fully blocked by a peptide inhibitor of TFPI.

Results: TFPI inhibition strongly improves thrombin generation at low TF in hemophilic and normal blood and plasma. Mean peak thrombin increased 7-fold in hemophilic plasma (21 – 151 nM) and 2.8-fold in normal plasma (83 – 229 nM). Triggering thrombin generation with 20 pM TF minimally increased peak thrombin (304 – 382 nM) in the absence of TFPI and FVIII. An even smaller increase in peak thrombin was observed in normal plasma upon TFPI inhibition. In whole blood assays, a similar TFPI effect decrease with increasing TF was observed. Clot time dropped from 6.5- to 3-fold when TFPI activity was blocked.

Conclusion: Coagulation triggered by high TF overrides the effect of TFPI inhibition independent of FVIII. Thus, TFPI inhibition might protect hemophilia target tissues where TF expression is low and most likely does not interfere in tissues protected by high TF.

Disclosure of Interest: R. Hartmann Employee of: Baxter Innovations GmbH, E. Panholzer Employee of: Baxter Innovations GmbH, W. Kammlander Employee of: Baxter Innovations GmbH, F. Scheiflinger Employee of: Baxter Innovations GmbH, M. Dockal Employee of: Baxter Innovations GmbH.

PO210-WED

Rare alternate presentations of the F8 intron 22 inversion mutation complicate detection and may lead to misdiagnosis

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Background: The F8 intron 22 inversion causes almost 50% of severe haemophilia A cases. There are several variations of this mutation, which presents challenges in its detection and assessment of clinical significance. There are several detection methods, all of which require good quality DNA: UKNEQAS returns indicate that half of laboratories use the Inverse PCR technique. It is known that variant inversion presentations give different results with this technique, and two independent primer sets were designed to overcome this limitation. However, some rare variant forms may give conflicting results, making conclusive identification difficult.

Aims: We report two families in whom discrepant results were obtained by inverse PCR. Family 1 comprises mother and son, and Family 2 consists of two male cousins.

Methods: Samples were digested, ligated and amplified with three independent primer sets, defined as 2005, 2008-diagnostic, and 2008-complementary primers.

Results: Using the 2005 primers, three affected males and one carrier female were identified, each amplifying product of expected 559 bp. The 2008-diagnostic primers failed to amplify a product from any of the male DNAs. The female sample amplified a single, wild type, band of 487 bp, producing a 'false negative' result. Using the 2008-complementary primers, a single 405 bp product was amplified for the affected male in family 1, representing amplification of the *int22 h-3*, and suggesting a type 2 inversion. His mother produced three bands consistent with her carrier status. The males in family 2 generated products of 559, 457 and 405 bp suggestive of a benign duplication, but lack of amplification with the diagnostic primers exclude this from being a classic duplication.

Conclusion: The number of pathogenic variant *F8* intron 22 presentations continues to expand, with two new types presented here. Failure to amplify with a single set of primers must be treated with caution: not necessarily indicative of problems with DNA quality, but maybe a previously unseen variant.

Disclosure of Interest: None Declared.

PO211-WED

Characterization of IGA type FVIII inhibitor in an adult patient with congenital moderate hemophilia A showing abnormally large subcutaneous hematoma similar to bleeding in patients with acquired hemophilia

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Background: We examined an adult patient with congenital moderate hemophilia A who showed abnormally large subcutaneous hematoma similar to bleeding experienced in patients with acquired hemophilia. FVIII inhibitor was demonstrated in the patient.

Aims: To clarify the abnormal bleeding, we precisely characterized the FVIII inhibitor.

Methods: The patient was a 66 years old male who had been diagnosed with congenital moderate hemophilia A (FVIII:C 4%). He had been suffering from polymyositis since the age of 63, and he had been treated with prednisolone (PSL) 5 mg day⁻¹ and azathioprine. A cardiac pacemaker was implanted as treatment for sick sinus syndrome with using recombinant FVIII concentrate. Six months after of the implantation, he showed abnormally large subcutaneous hematoma accompanied with anemia. 1.8BU of FVIII inhibitor was demonstrated, and the level of his FVIII:C was undetectable. To eradicate the inhibitor, 60 mg day⁻¹ of PSL was initiated. FVIII inhibitor was dramatically undetectable after 3 weeks of this treatment.

Results: Western-Blotting (WB) analysis revealed that the FVIII inhibitor consisted of polyclonal IgG, IgM and predominantly IgA, and their specificity were for the heavy chain of procoagulant FVIII, but not to the A1 and A2 domains of coagulant FVIIIa. Furthermore, a synthetic peptide derived from 337-353aa in the a1 region (337-372aa) of FVIII showed an inhibitory effect on the binding of the IgA type FVIII inhibitor in WB analysis, and also dose-dependently decreased the FVIII inhibitor titer in BU assays.

Conclusion: These results suggest that the epitope of the rare IgA-type FVIII inhibitor is in the vicinity of 337-353aa of the acidic a1 region of FVIII molecule. Binding of IgA to this region may interfere with the cleavage reaction of a1 and A2, which is essential in engaging FIXa and is extremely important for FVIII activation. However, the clinical association between the appearance of the IgA-type FVIII inhibitor and the development of the abnormal bleeding remains unclear.

Disclosure of Interest: None Declared.

PO212-WED

Recombinant factor VIII increases hemophilia A clot strength through alterations in clot structure and composition

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Background: Hemophilia A (HemA) is a bleeding disorder caused by factor VIII (FVIII) deficiency, and FVIII replacement is the standard of care. The relationship between thrombin levels and clot structure and viscoelasticity in normal blood is well established.

Aims: To investigate the effects of FVIII on in vitro clot structure and viscoelastic properties and on clots formed by FVIII-treated HemA mice subjected to vascular injury

Methods: In vitro HemA clots were analyzed by confocal microscopy and rheometry. *In vivo* studies were performed on anesthetized HemA mice dosed with rFVIII to achieve desired levels (5%–100%), followed by saphenous vein puncture and primary clot formation. Primary clots were further disrupted to generate secondary clots. Bleeding times associated with primary and secondary clot formation were measured, and vein segments containing clots were harvested and processed for scanning electron microscopy. Wild-type C57B6 mice served as controls for HemA mice in the studies.

Results: FVIII had striking effects on in vitro HemA clot structure and viscoelasticity, with maximal effect at the 25% level, including greatly increased branching and higher stiffness. *In vivo*, FVIII affected secondary more than primary clot formation, achieving minimum clot times at ~25% plasma FVIII. Saphenous vein clots contained, in addition to fibrin and platelets, closely packed polyhedrocytes. HemA clots formed at <25% plasma FVIII contained fewer fibrin- and erythrocyte-derived structures but higher platelet content. Clots formed in the presence of ≥25% plasma FVIII had similar composition as those derived from wild-type mice: >40% fibrin-, ~12% platelet-, and ~45% erythrocyte-derived structures.

Conclusion: Taken together, our results indicate that lower (<100%) FVIII levels may be sufficient for hemostasis, and that bleeding in hemophilia may be a function of both plasma FVIII and clot architecture and properties.

Disclosure of Interest: L. Leong Employee of: Bayer HealthCare, I. Chernysh: None Declared, Y. Xu Employee of: Bayer HealthCare, D. Sim Employee of: Bayer HealthCare, C. Nagaswami: None Declared, Z. de Lange: None Declared, S. Kosolapova: None Declared, A. Cuker: None Declared, K. Kauser Employee of: Bayer HealthCare, J. Weisel Grant/Research Support from: Bayer HealthCare.

PO213-WED

Adding multiple polyethylene glycol (PEG) moieties further reduces PEG-factor VIII conjugate (PEG-FVIII) clearance by hepatocytes in vitro, but minimally impacts PEG-FVIII clearance or half-life *in vivo*Blasko E¹, Ho E¹, Wu J¹, Sim D², Tang L¹, Leong L², Kauser K² and Subramanyam B¹¹Biologics Research; ²Hematology Research, Bayer Healthcare LLC, San Francisco, USA

Background: Site-specific PEG conjugation of FVIII reduced *in vivo* clearance and prolonged half-life, partly through PEG steric hindrance of PEG-FVIII interactions with hepatocytes. As some di-PEG conjugates of FVIII have reduced specific activity (Mei et al., Blood 116: 270, 2010), perhaps also through steric effects, adding more PEG moieties to FVIII could further reduce its clearance.

Aims: Ascertain 1) whether the number of PEG moieties conjugated to FVIII affects PEG-FVIII clearance and 2) whether disruption of PEG-FVIII binding to membrane heparan sulfate proteoglycan (HSPG), one of the initial steps in FVIII internalization and degradation, accounts for reduced *in vitro* clearance.

Methods: Clearance of B-domain deleted (BDD)-FVIII, and its site-specific conjugates (mono-PEGylated PEG-FVIII or di-PEGylated (PEG)₂-FVIII) was assessed by *in vitro* hepatocyte clearance assay and by pharmacokinetic studies in HemA mice. Clearance studies were also performed in the presence of heparin, a competitor of HSPG, to determine whether reduced PEG-FVIII binding to HSPG may contribute to reduced PEG-FVIII clearance.

Results: Mono-PEGylated FVIII showed reduced clearance *in vitro* relative to BDD-FVIII (139%), and the clearance of a di-PEGylated (PEG)₂-FVIII was reduced even further (202%). Heparin reduced clearance of all three proteins, consistent with published reports of HSPG binding preceding and facilitating FVIII binding to the LRP receptor. However, *in vivo* clearance of both PEG-FVIII and the (PEG)₂-FVIII tested were similarly reduced (204% and 211%, respectively) relative to BDD-FVIII, indicating that membrane interactions other than HSPG may contribute to PEG-FVIII *in vivo* clearance and half-life.

Conclusion: These results indicate the benefit of single PEG conjugation in reducing clearance. However, the data also demonstrate potential limitation of conjugating additional PEG moieties to FVIII to further reduce clearance.

Disclosure of Interest: None Declared.

PO214-WED

Distribution of 14C-labeled PEG (60 kDa) in rats

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Background: BAY 94-9027, a B-domain-deleted recombinant FVIII with one 60 kDa polyethylene glycol (PEG; 2 × 30 kDa branched) attached via a linker to cysteine 1804 (ie, site-specific PEGylation), is in clinical development for acute and prophylactic treatment of patients with hemophilia A. While there is no clinical evidence suggesting long-term safety issues related to the use of PEGylated proteins, questions have been raised regarding the pharmacokinetics (PK) including metabolism and distribution particularly for PEGylated proteins used chronically and/or in the pediatric population.

Aims: To investigate the distribution of residual radioactivity (unchanged compound and radioactive metabolites) on Days 7–168 after single intravenous (IV) administration of [¹⁴C] BAY 1025662, the cysteine-linker-PEG 60 kDa component of the molecule containing a covalently linked [¹⁴C] label in the linker, in male Wistar rats

Methods: The single IV dose was 11 mg kg⁻¹, the approximate human lifetime dose of PEG 60 kDa from treatment with BAY 94-9027. Con-

centrations and amounts of radioactivity in organs and tissues were measured using whole body autoradiography 6 months after administration.

Results: Between Days 7–168 post-injection, radioactive residues in the body decreased steadily from 22.5% of the dose to 1.8%. A heterogeneous distribution of radioactive residues was observed, with apparent affinity of radioactivity to endocrine and exocrine glands and lymphatic organs. No residual radioactivity was detected in brain and adipose. Elimination was fairly complete, with a total recovery of radioactivity of 94% at Day 168.

Conclusion: After single-dose administration to rats of the estimated human lifetime dose of PEG resulting from BAY 94-9027 treatment, radioactive residues in the animal body decreased steadily and elimination was fairly complete. Terminal elimination occurred slowly, but there was no indication of retention or irreversible binding of radioactivity in the animal body.

Disclosure of Interest: A. Baumann Employee of: Bayer Pharma AG, T. Schwarz Employee of: Bayer Pharma AG, D. Seidel Employee of: Bayer Pharma AG, W. Steinke Employee of: Bayer Pharma AG, K. Buehner Employee of: Bayer Pharma AG.

PO215-WED

Single center experience evaluating activity of N8-GP with one-stage clotting assayBrand-Stauber BC¹, Koch K¹, Balabanov S¹, Clausen WHO² and Ezban M³¹Division of haematology, University Hospital Zurich, Zurich, Switzerland; ²Biostatistics, Biopharm, Novo Nordisk A/S, Søborg; ³Haemophilia Biology, Novo Nordisk A/S, Måløv, Denmark

Background: Modified factor VIII products with extended half-life are tested in clinical trials or reaching the market. The one-stage clotting assay (OS) is the most widely used assay for monitoring of patients. Currently only a minority of laboratories are using chromogenic assays (CA). The OS is a biologic assay with inherent variation due to the large variety of aPTT reagents, instrumentations and calibrators used. This variability and complexity is being increased with new modified products. N8-GP is a GlycoPEGylated recombinant FVIII in clinical development. The PEG moiety interferes with some aPTT reagents used in OS. Careful assessment of the accuracy and precision of the assays used for therapeutic monitoring is therefore necessary to support the evaluation of the efficacy and safety of these new long acting factor products.

Aims: Analysis of performance of OS factor VIII assays in monitoring trough and peak levels in patients treated with N8-GP, comparison of results when using a product calibrator provided by Novo Nordisk and the laboratory's standard plasma calibrator.

Methods: Analysis of all available factor VIII OS values obtained with and without product specific standard from patients in Novo Nordisk trial NN7088-3859 between 06/2012 and 01/2014. Laboratory equipment: BCS XP Systems, Siemens Diagnostics. Reagents: deficiency plasma, aPTT reagent Pathromtin SL (five different lots), standard human plasma all by Siemens. Samples were tested in three dilutions.

Results: In total 89 measurements in three patients were analysed by Bland-Altman plot showing that the data correlate and no systematic difference were observed between the two local lab results. This observation was consistent and independent of used aPTT lots.

Conclusion: The described test modalities used in our center showed satisfying accuracy of the standard OS assay without product specific standard to control trough and post-infusion level of factor VIII treated with a PEGylated factor VIII molecule.

Disclosure of Interest: B. C. Brand-Stauber Consultant for: NovoNordisk, Bayer, K. Koch: None Declared, S. Balabanov: None Declared, W. H. O. Clausen Employee of: NovoNordisk, M. Ezban Employee of: NovoNordisk.

PO216-WED

International study to assess the performance of immunodepleted plasmas for determination of factors VIII and IX activity

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Background: Accurate and reproducible measurement of Factor VIII (FVIII) or Factor IX (FIX) activity is essential for assessing haemophilia.

Aims: To assess the performance of two novel immuno-depleted FVIII and FIX deficient human plasma products in the one-stage FVIII and FIX activity assay in three international laboratories.

Methods: FVIII and FIX activity results obtained on the STA-R® analyzer using the new STA® - ImmunoDef VIII & IX products (ImmunoDef.) were compared to results obtained using current STA® - Deficient VIII & IX products (Def.). Calibrations were run using STA® - Unicalibrator and STA® - System Control N + P were tested as Internal Quality Control (IQC).

For FVIII activity N = 474 samples, including N = 207 haemophilia A patient samples, were tested. For FIX activity N = 399 samples, including N = 138 haemophilia B patient samples, were tested. Samples covered the working range of the new products (i.e. 0.7-400% for FVIII and 0.7%>300% for FIX).

Correlations were analysed using Passing Bablok regression. Bias was calculated and assessed at the haemophilia decision levels: 1% and 5% (expressed as absolute bias) and 40% (expressed as relative bias).

ImmunoDef. VIII & IX's results obtained with a daily calibration were compared to results obtained with the valid calibration of the first day (rerun only if IQCs were out of range).

Results: Results are similar when calculated with the daily calibration or the valid calibration.

Passing Bablock regressions were as follow: $y = 0.98x + 1.52$ for FVIII and $y = 1.08x - 0.73$ for FIX.

The bias at the clinical decision levels for hemophilia were as follows:

FVIII: 0.2% at 1%; 0.2% at 5% and 6.3% at 40%

FIX: 0.0% at 1%; 1.0% at 5% and 12.4% at 40%

Conclusion: Equivalent results between ImmunoDef. and Def. products were obtained for both FVIII and FIX. The results also show that there is no requirement for a daily calibration and confirm that haemophilia patients can be accurately classified with the new products.

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PO217-WED

Genetic determinants for factor VIII levels: genome-wide linkage and association analyses from the GAIT project

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Background: Plasma factor VIII (FVIII) levels are highly heritable and strongly associated with thrombosis risk. The genetic factors that predispose to elevated FVIII are unknown.

Aims: We aimed to perform a genome-wide linkage analysis and fine-mapping to delineate the genomic regions that influence the variance in FVIII levels in Spanish families from the Genetic Analysis of Idiopathic Thrombophilia Project (GAIT1 and GAIT2).

Methods: Functional clotting FVIII (FVIII:C) was measured in plasma of 398 subjects belonging to 21 families (GAIT1). A variance component linkage method with 485 DNA microsatellite markers was used to evaluate linkage and to detect quantitative trait loci (QTLs). Fine-mapping with 2593 single nucleotide polymorphisms (SNPs) was conducted in the genomic regions containing linkage signals to find out SNPs associated with FVIII levels. Replication of the association analysis in an independent sample was performed by using 935 subjects from 35 new GAIT families (GAIT2).

Results: Two QTLs, located on Chromosome 2 and Chromosome 3, showed strong evidence of linkage with FVIII levels. The gene *CIB4* on Chromosome 2 and *ARHGEF3* and *ADAMTS9* on Chromosome 3 were positional genes that could influence FVIII levels. Fine-mapping in these linkage regions revealed one intron-variant in the *CIB4* gene on Chromosome 2 significantly associated with FVIII levels. No association was found on Chromosome 3, suggesting that a rare variant may contribute to the linkage signal.

Conclusion: We report two new QTLs on Chromosomes 2 and 3 involved in quantitative variation of plasma FVIII levels. On Chromosome 2, one SNP in *CIB4* was associated with FVIII levels, suggesting a functional effect. Our results shed light on the genetic factors that contribute to the heritability of FVIII and could help to elucidate the biological mechanisms underlying the variance in FVIII levels and thrombosis risk.

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Disclosure of Interest: None Declared.

PO218-WED

Quantitation of misfolded proteins and quantitation and visualisation of nanosize protein aggregates in rFVIII and PDVIII concentrates

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Background: Changes in protein structure leading to protein aggregation is a prevalent problem occurring in many biopharmaceutical manufacturing processes. These aggregates are believed to be harmful due to an increased inflammatory and immunogenicity potential.

Aims: As the presence of aggregates in biopharmaceutical formulations remains a quality and safety concern we analyzed the amount of misfolded proteins and of sub-visible protein particles in rFVIII and pdVIII concentrates.

Methods: Misfolded proteins in FVIII concentrates were quantified by binding to the chaperone GRP78/BIP, and to the multi-ligand receptors for misfolded proteins tPA, and specific peptides from kringle 5 domain of plasminogen as well as metalloprotease domain of ADAM15 using Elisa technique. Particles < 1000 nm were examined in three rFVIII products (Kogenate® Bayer, Advate Baxter, ReFacto AF Pfizer) and five pdFVII products (Octanate® Octapharma, Haemocin SDH Biotest, Fanhdi® Grifols, Beriate P CLS Behring, Immunate STIM plus Baxter) using a NanoSight Nanoparticle tracking analysis (NTA) system.

Results: All pdFVIII products contained misfolded albumin. In all pdFVIII concentrates with the exception of Fanhdi, large amounts of misfolded fibrinogen were found. The low level of misfolded fibrinogen reflects the low level of fibrinogen in Fanhdi. None of rFVIII products did contain misfolded fibrinogen. The amount of misfolded FVIII was higher in plasma derived FVIII products, but all FVIII products contained probably some misfolded FVIII molecules.

While the rFVIII contained only few sub-visible protein particles (1 to 6×10^8), many particles from different sizes were found in all tested pdFVIII samples (12 to 45×10^8).

The size distribution of particles in the different concentrates differed between the products.

Conclusion: PdFVIII products contain lots of misfolded proteins. This has been demonstrated by sub-visible protein particles as well as by strong binding of proteins inside pdFVIII concentrates to 'misfolding probes'.

Disclosure of Interest: None Declared.

PO219-WED

Heparosan glyco-conjugates of FVIII and fix shows prolonged circulating half-life in haemophilia mouse models

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Background: Several strategies for increasing the half-life of recombinant FVIII (rFVIII) and FIX (rFIX) are currently being pursued to improve prophylaxis in haemophilia. One concept involves polymer conjugation. Among several polymers evaluated, heparosan was selected as an alternative polymer for conjugation to rFVIII and rFIX. Heparosan is a linear polysaccharide containing repeating units of glucuronic acid (GlcUA) and N-acetylglucosamine (GlcNAc).

Aims: Evaluate the possibility of increasing circulating half-life of rFVIII and rFIX by conjugation to heparosan.

Methods: 40 kDa-heparosan was conjugated to an O-linked glycan in the B-domain of rFVIII (turoctocog alfa) and to an N-linked glycan in the activation peptide of rFIX (ref 1, 2). Pharmacokinetics were evaluated after iv administration of 140 IU kg⁻¹ rFVIII to F8-KO mice and 290–360 IU kg⁻¹ rFIX to F9-KO mice. Blood samples were collected in a sparse sample design and plasma concentrations of rFVIII and rFIX were determined using chromogenic assays (Coatest® SP, Chromogenix, and Biophen® FIX, Hyphen Biomed).

Results: SDS-PAGE and reversed phase- and size exclusion-HPLC demonstrated heparosan conjugation at the expected glycans. Upon activation, native FVIIIa and FIXa were generated. In F8-KO mice,

the 40 kDa-heparosan conjugated rFVIII had a terminal half-life of 14 h, compared to 8 h for rFVIII (Advate®). Similarly, 40 kDa-heparosan-conjugated rFIX had a terminal half-life of 34 h in F9-KO mice compared to 14 h for rFIX (Benefix®).

Conclusion: Heparosan-conjugation resulted in 1.8-fold prolonged half-life of rFVIII and 2.5-fold prolonged half-life of rFIX potentially enabling prophylaxis in haemophilia with less frequent dosing and/or higher through levels.

Ref 1) Stennicke HR, *et al.* Blood. 2013, 121(11), 2108-2116. 2) Østergaard H *et al* Blood, 2011, 118 (8), 2333-2341.

Disclosure of Interest: C. Behrens Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, J. Buchardt Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, M. Broberg Employee of: Novo Nordisk A/S, D. Karpf Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, M. Loftager Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, M. Jensen Employee of: Novo Nordisk A/S, H. Bagger Employee of: Novo Nordisk A/S, M. Thóroldsson Employee of: Novo Nordisk A/S, M. Dainiak Employee of: Novo Nordisk A/S, J. Karlsson Shareholder of: Novo Nordisk A/S, E. Hansen Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, J. Krarup Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, H. Rahbek-Nielsen Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, M. Kjalke Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, P. DeAngelis Employee of: Caisson Biotech, LLC, F. M. Haller Employee of: Caisson Biotech, LLC, J. Haaning Employee of: Novo Nordisk A/S.

PO220-WED

Suppression of inhibitor formation in hemophilia by LAP+ TREG induced by plant-based oral tolerance

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Background: Recently, we have developed oral tolerance protocols for hemophilia A and B based on delivery of CTB fusion antigens expressed in transgenic chloroplasts and bioencapsulated in leaf cells of crop plants (PNAS 107:7101,2010; Blood 124:1659, 2014).

Aims: To define the mechanism of plant-based oral immune tolerance, including the role of immune regulatory cells and pathways.

Methods: Hemophilia B mice (C3H/HeJ f9/- or C3H/HeJ f9/- x il-10/-) received 2 months of oral gavages (twice/week) of plant cells containing 5 ug factor IX (FIX) antigen. FIX replacement therapy (1 IU, IV, once/week) was performed for 2 months, starting 1 month into the oral regimen.

Results: Oral tolerance suppressed inhibitor formation (ave. of 2 vs 18 BU, $P < 0.01$) as well as and IgE formation/anaphylaxis. The combination of oral and systemic delivery of FIX caused a marked increase in the frequencies of CD103⁺ dendritic cells (DC) and plasmacytoid DC in mesenteric lymph nodes (MLN) and Peyer's patches (PP). In addition, CD4 + CD25-FoxP3-LAP+ T cells were robustly induced in PP (from <5% to 10-20%), and to lesser extent in spleen and MLN. These cells expressed IL-10 and TGF-b in response to FIX stimulation. Adoptive transfer studies confirmed induction of CD4 + CD25-LAP- Treg that suppressed anti-FIX formation. Induction and suppression was antigen-specific and unsuccessful in IL-10 deficient mice.

Conclusion: Treg expressing latency-associated peptide(LAP), known to suppress through a TGF-b dependent mechanism, are distinct from CD4 + CD25 + FoxP3 + cells and can potently suppress inhibitor formation upon oral antigen delivery. Induced LAP+ Treg upregulate immune suppressive cytokines IL-10 and TGF-b in response to antigen, and their induction is IL-10 dependent.

Disclosure of Interest: None Declared.

PO221-WED

Low-density lipoprotein receptor-related protein 1 expression in liver is associated with plasma factor VIII levels

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Background: Factor VIII (FVIII) is a crucial key in the coagulation cascade and its deficiency or increased levels are associated to important diseases as hemophilia A and venous thromboembolism, respectively. The low-density lipoprotein receptor-related protein 1 (LRP1) is a multifunctional receptor which plays an important role in endocytosis and catabolism of its ligands, including the FVIII.

Aims: The aim of the study was investigate the LRP1 protein expression in liver cells subtypes of healthy individuals submitted to cholecystectomy or gastropasty. Moreover, we evaluate the association between this protein expression and plasma FVIII level.

Methods: Adult healthy liver biopsies were obtained from 20 subjects (all female), with a median age of the 49 years undergoing cholecystectomy or gastropasty between January-June 2013. Their medical records were analyzed and those with a history of haemostatic disease, cancer, renal or hepatic insufficiency, inflammatory or infectious disease were excluded. From each individual, we drawn blood sample at three time points: pre-operative (T = 0), intra-operative (T = 1), and 12 h after surgery (T = 2). LRP1 expression in the liver cells were evaluated by digestion with collagenase solution followed by flow cytometry analysis, and FVIII activity levels were measured by a one-stage clotting assay. This study was approved by the local Ethics Committee on Human Research and a written informed consent was obtained from all study participants.

Results: We demonstrated an inverse correlation between LRP1 expression in all cells (hepatocytes+endothelial+Kupffer cells) evaluated and plasma FVIII levels at T = 1 ($\rho = -0.50$; $P = 0.03$), which was not associated with a specific cell subtype.

Conclusion: The increase of plasma FVIII levels after stimulus as anesthesia and surgery are well established. Our results support the hypothesis that the expression of hepatic LRP1 modulates this response, since we observed an inverse correlation between hepatic LRP1 expression and plasma FVIII levels.

Disclosure of Interest: None Declared.

PO222-WED

The immunogenicity of platelets containing FVIII in hemophilia a mice with or without pre-existing anti-FVIII immunity

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Background: Evidence suggests that platelet-derived FVIII (Plt-F8) is therapeutic in F8^{-/-} mice even with anti-F8 inhibitory antibodies (inhibitors). If current efforts to generate platelets *in vitro* succeed, genetically manipulated platelets containing FVIII may be used therapeutically in hemophilia A (HA) patients even with inhibitors.

Aims: The aim of this study is to explore the immunogenicity of Plt-F8.

Methods: Platelets from 2bF8 transgenic (2bF8^{tg}) mice in which FVIII expression is targeted to platelets were infused into F8^{-/-} mice with or without inhibitors weekly. Total and inhibitory anti-F8 antibodies (Abs) were determined by ELISA and Bethesda assay respectively.

Results: To investigate whether Plt-F8 can act as an immunogen in F8^{-/-} mice, we infused 2bF8^{tg} platelets into naïve F8^{-/-} mice without preconditioning to a level of 20-57% weekly for 8 weeks. No anti-F8 Abs were detected in infused mice during the study course. All animals developed inhibitors following further challenge with rhF8. We then explored if Plt-F8 can act as an immunogen in F8^{-/-} mice with inhibitors. F8^{-/-} mice were immunized with rhF8 to induce anti-F8 Abs. After inhibitor model was established, 2bF8^{tg} platelets were transfused weekly for 4 weeks. There was no augmentation of F8-specific Abs. To investigate if preconditioning affects the anti-F8 immune response, animals were sub-lethally irradiated with 660 cGy followed by 2bF8^{tg} platelet infusion weekly for 8 weeks. No anti-F8 Abs were detected in recipients after platelet infusion. Following further challenge with rhF8, the inhibitor titer in this group was significantly lower (75 ± 42 BU mL⁻¹) than in the naïve F8^{-/-} mice utilizing the same infusion protocol but without preconditioning (270 ± 76 BU mL⁻¹), indicating that 660 cGy conditioning plus 2bF8^{tg} platelet infusion may suppress anti-F8 immune response.

Conclusion: Our data demonstrate that infusion of Plt-F8 triggers neither primary nor memory anti-F8 immune response in HA mice.

Disclosure of Interest: None Declared.

PO223-WED

Elevated factor VIII levels and all-cause mortality: results from the mega follow-up study

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Background: Elevated factor VIII levels are an established risk factor for venous thrombosis and to a lesser degree, for arterial thrombosis as well. Elevated factor VIII levels have also been associated with an increased risk of death, although results are contradictory.

Aims: We aimed to explore whether elevated factor (F) VIII levels are associated with an increased risk of death in patients who had venous thrombosis as well as in individuals from the general population and to which extent this association is causal.

Methods: We performed a follow-up study of patients and control individuals from the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis study (MEGA study) in which in more than half of the participants FVIII levels were measured. 2178 patients with previous venous thrombosis and 2827 age and sex matched community controls in whom FVIII and ABO blood group were measured were followed up for on average 5.5 years.

Results: All-cause mortality rates increased in a dose-response fashion with increasing categories of FVIII levels. In the thrombosis patients, mortality risk was highest above the 97.5th percentile (FVIII > 199 IU dL⁻¹) with a hazard ratio (HR) adjusted for age, sex, body mass index and comorbidities of 3.1 (95% confidence interval [CI], 0.9-10.8) compared with patients in the 25th percentile category (FVIII ≤ 85 IU dL⁻¹). The adjusted HR was 4.5 (95%CI, 1.4-14.3) in controls. Using blood group non-O as a measure of genetically elevated FVIII levels to determine a causal relationship between FVIII and death showed HRs of 0.99 (95%CI, 0.72-1.36) in patients and 1.25 (95%CI, 0.82-1.90) in controls.

Conclusion: We showed a dose-response relationship between high FVIII levels and risk of death in venous thrombosis patients and in individuals from the general population. However, because individuals with non-O blood group did not have an increased risk of death, the relation is probably not causal.

Disclosure of Interest: None Declared.

PO224-WED

Novel mutations associated with a discrepancy between one-stage and chromogenic FVIII activity assay

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Background: FVIII activity (FVIII:C), measured in different ways is identical for most hemophilic patients. However, there is a significant number of mild/moderate hemophiliacs who show different FVIII:C phenotype depending on a certain type of causal mutations.

Aims: To find and compare the prevalence of FVIII:C discrepant patients with mild/moderate hemophilia (HA) in the Czech Republic. To characterize the mutation profile of patients with discrepant values of FVIII:C measured by one-stage (FVIII:C_{1st}) and chromogenic assay (FVIII:C_{Chr}).

Methods: We examined FVIII:C in 76 patients with mild/moderate HA using both methods. Our group of patients included 14 moderate and 62 mild hemophiliacs measured with FVIII:C_{1st}. The obtained results were compared and declared as different when the proportion of FVIII:C_{1st}/FVIII:C_{Chr} or FVIII:C_{Chr}/FVIII:C_{1st} was ≤ 0.6 . Causal mutations of the *F8* gene were assigned to patients with FVIII:C discrepancies.

Results: Based on the stated criteria, we found out 15 analyzed patients with significantly different values of FVIII:C_{1st} and FVIII:C_{Chr}. FVIII:C_{1st} was higher in 11 patients; three patients had even FVIII:C_{1st} > 40 IU dL⁻¹, while FVIII:C_{Chr} was on average 16 IU dL⁻¹ (12–23 IU dL⁻¹). Genotyping of hemophiliacs with discrepant FVIII:C detected 11 novel mutations not associated with such a discrepancy to date. Mutations in patients with a lower FVIII:C_{1st} were localized to the A2 domain and in those with a lower FVIII:C_{Chr} predominantly to the A3 domain.

Conclusion: Our results show that the FVIII:C value is dependent on the method used in 20% of non-severe HA patients in the Czech Republic, which is a lower figure than in other countries (30% in Germany and Great Britain, 50% in Denmark). This could be explained by a different mutation profile associated with a discrepancy of the Czech mild/moderate hemophiliacs comparing with other countries.

Disclosure of Interest: None Declared.

PO225-WED

Hemophilia A caused by retrotransposition of long interspersed element-1 (LINE-1) into the FVIII gene

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Background: A patient with no family history of bleeding disorders was diagnosed with severe hemophilia A (FVIII < 1%) due to post-circumcision bleeding on day 8.

Aims: To identify the mutation causing hemophilia A in the above patient.

Methods: The patient was found to be negative for the common mutations, intron 22 and intron 1 inversions. Therefore, all the 26 exons and exon/intron boundaries of the FVIII gene were directly sequenced following PCR amplification.

Results: The fragment obtained using primers encompassing exon 23 was larger than expected by about 800 bp. Sequence analysis of the mutant exon 23 revealed an insertion of 616 bp perfectly matching a fragment of LINE-1 sequence (GenBank accession no. JN698885.1) in anti-sense orientation, preceded by a poly-T stretch of about 180 bp,

which could not be precisely sequenced. The insertion was accompanied by target duplication of 17 bp, which is a typical feature of LINE-1 element insertion. Analysis of the patient's mother revealed only the normal exon 23 sequence, suggesting that she is not a carrier of this mutation. Using quantitative PCR to amplify a hybrid fragment specific for the insertion, encompassed by one primer derived from the inserted sequence and a second primer derived from exon 23 of the FVIII gene, we confirmed the absence of patient's mutation in his mother's DNA. However, germ cell mosaicism cannot be excluded.

Conclusion: The molecular defect causing hemophilia A in this patient is an insertion of a reverse-transcribed LINE-1 fragment into exon 23 of the FVIII gene, which probably occurred in one or more of his mother's germ cells or during fetal development. To the best of our knowledge, only three cases of hemophilia A resulting from LINE 1 insertion into the FVIII gene have been published to date (exon 14, 2 patients [Kazazian et al., 1988]; together with a large deletion spanning exons 15–20, 1 patient [Van de Water, 1998]). Thus, it is a relatively rare mechanism of mutagenesis among hemophilia A patients.

Disclosure of Interest: None Declared.

PO226-WED

Complement modulates the immune response against therapeutic factor VIII in hemophilia A

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Background: Injection of exogenous factor VIII (FVIII) to treat or prevent bleeding in patients with hemophilia A is associated, in up to 30%, with the occurrence of inhibitory anti-FVIII antibodies. Several recent studies have highlighted a strong interplay between the molecules of the complement system and the coagulation cascade. Besides, complement is known to participate in the initiation of immune responses against pathogens.

Aims: Investigate the role of the complement system in the onset of the anti-FVIII immune response.

Methods: Complement was depleted in FVIII-knock out (KO) mice by injecting humanized cobra venom factor during a FVIII immunization protocol. Anti-FVIII antibodies levels were titrated by ELISA and inhibitor assays. We further studied the effect of complement on the uptake of FVIII by human monocyte-derived dendritic cells (Mo-DCs) generated from healthy donors, on the maturation state of Mo-DCs and on the presentation of FVIII by Mo-DCs to a FVIII-specific mouse CD4⁺ T-cell hybridoma. Human AB serum was used as a source of complement.

Results: Complement depletion in FVIII-KO mice drastically impaired the anti-FVIII humoral response. FVIII uptake by Mo-DCs was significantly lower in the presence of heat-inactivated or C3-depleted serum as compared to normal serum. The reduced FVIII uptake resulted in a decreased activation of FVIII-specific T cells. Complement had no effect on the maturation of Mo-DCs. Together, our data suggest that complement is involved in the anti-FVIII immune response by facilitating the uptake of FVIII by Mo-DCs.

Conclusion: Our work should pave the way towards a better understanding of the pathogenesis of the anti-FVIII immune response and the crosstalk that exist between the complement system, the coagulation cascade and the immune system.

Disclosure of Interest: None Declared.

PO227-WED

Surface bound von willebrand factor modulates factor VIII peptide presentation by dendritic cells

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Background: A major complication in hemophilia A treatment is the development of neutralizing antibodies against human coagulation factor VIII (FVIII). FVIII circulates as a complex in plasma with von Willebrand Factor (VWF). Several studies propose that VWF also affects FVIII immunogenicity by reducing FVIII uptake by dendritic cells (DCs).

Aims: The aim of this study is to determine the interaction with and processing of VWF by dendritic cells. We also studied the role of VWF in FVIII endocytosis and MHC class II presentation by DCs.

Methods: Human monocyte-derived DCs were incubated with FVIII, VWF or FVIII/VWF complex. Binding/uptake was analyzed by flow cytometry and confocal microscopy. HLA-DR-peptide repertoire of the pulsed DCs was determined using mass spectrometry.

Results: Interestingly, FACS and confocal analysis revealed that VWF is not internalized by DCs but remains tightly bound to the cell surface. Consistent with these findings we did not observe presentation of VWF-derived peptides on MHC class II. In accordance with previous findings uptake of FVIII was reduced in the presence of VWF. Under these conditions FVIII derived-peptides were still abundantly presented on MHC class II suggesting that efficient peptide presentation of FVIII does not depend on the amount of antigen endocytosed. Inspection of peptide profiles, revealed differences in FVIII core sequences in samples treated with FVIII/VWF when compared to FVIII alone. This finding shows that VWF modulates MHC class II peptide presentation of FVIII. A small number of VWF derived peptides were identified when cells were incubated with FVIII/VWF complex, suggesting that FVIII endocytosis might promote or facilitate uptake of small amounts of VWF by DCs.

Conclusion: Our results show that VWF is not endocytosed or presented by DCs unless in complex with FVIII. We also demonstrate that VWF modulates MHC class II peptide presentation of FVIII thereby potentially affecting its immunogenicity.

Disclosure of Interest: None Declared.

PO228-WED

Role of membrane-interacting residues within factor VIII C2 domain in immunogenicity of therapeutic factor VIII

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Background: Twenty to 30% of severe hemophiliacs develop antibodies to therapeutic factor VIII (FVIII). Our understanding of immune recognition and cellular mechanisms involved in FVIII uptake is however limited. We previously demonstrated that the human monoclonal C2 domain-specific antibody BO2C11 inhibits FVIII uptake *in vitro* and is protective *in vivo*.

Aims: Investigate the importance of basic residues within BO2C11 epitope in FVIII uptake *in vitro* and immunogenicity *in vivo*.

Methods: FVIII, FVIII^{C1}, FVIII^{C2} or combined FVIII^{C1C2} mutants were expressed and purified. *In vitro* uptake assays were performed

using monocyte-derived dendritic cells (MODCs) from healthy donors, bone marrow-derived dendritic cells from FVIII-knockout (KO) mice, and low-density lipoprotein receptor (LRP) overexpressing cell line (U87MG). *In vivo* immunization studies were performed upon weekly intravenous administration of 1 µg purified FVIII or FVIII mutants in FVIII-KO or FVIII/von Willebrand factor (VWF)-KO mice. Anti-FVIII antibody titers were measured using ELISA or inhibitor assays.

Results: FVIII^{C2} mutants exhibited diminished uptake *in vitro* by MODCs, BMDCs and U87MG cells as compared to wild-type FVIII, as previously published for C1 mutants. However, the *in vivo* immunogenicity of FVIII^{C1}, FVIII^{C2} or FVIII^{C1C2} mutants was not reduced in FVIII-KO mice. Interestingly, FVIII^{C1} or FVIII^{C1C2} exhibited significantly reduced immunogenicity in FVIII/VWF-KO mice.

Conclusion: FVIII^{C2} mutants exhibited diminished uptake *in vitro* by MODCs, BMDCs and U87MG cells as compared to wild-type FVIII, as previously published for C1 mutants. However, the *in vivo* immunogenicity of FVIII^{C1}, FVIII^{C2} or FVIII^{C1C2} mutants was not reduced in FVIII-KO mice. Interestingly, FVIII^{C1} or FVIII^{C1C2} exhibited significantly reduced immunogenicity in FVIII/VWF-KO mice.

Disclosure of Interest: None Declared.

PO229-WED

FVIII activity assay variability of the pegylated form of RFVIII ADVATE (BAX 855) in clinical hemostasis laboratories: interim results from a world-wide field study

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Background: Analytical issues had been reported with modified FVIII. BAX 855 is a PEGylated recombinant human full-length FVIII based on the parent protein ADVATE.

Aims: Potency assignment of BAX 855 is performed with a 1-stage clotting assay relative to an in-house reference traceable against the WHO standard. Human FVIII-deficient plasma was spiked *in vitro* with BAX 855 or ADVATE at mathematical target concentrations of 0.80, 0.20 and 0.05 IU FVIII mL⁻¹, based on the labeled potencies. Samples were blinded for product and concentration and frozen aliquots were distributed to clinical hemostasis and diagnostic laboratories world-wide. Laboratories were asked to perform three independent assay runs using their routine FVIII activity assays.

Methods: All laboratories used 1-stage clotting assays with a variety of aPTT reagents and instruments, a small portion of laboratories also provided data from chromogenic assays. Twenty-three data sets from 1-stage clotting assays and nine data sets from chromogenic assays were available for this interim data evaluation.

Results: Preliminary data show similar intra- and inter laboratory variability for BAX 855 and ADVATE in the 1-stage clotting assays. *In vitro* recovery vs. mathematical target appears acceptable for both products. The percent relative error for ADVATE is slightly higher than for BAX 855, both show a trend to over-recovery at the low concentrations. With the chromogenic assays, between-lab variability is similar for both products; variability is highest at the lowest FVIII concentration. Geometric mean recovery is comparable for BAX 855 and ADVATE. The average ratio between the 1-stage clotting assay and the chromogenic assay was comparable although lab-to-lab variability was high.

Conclusion: BAX 855 in human plasma can be monitored with both 1-stage clotting assays and chromogenic assays using the routine methods established in clinical laboratories. There is no need for a product-specific standard.

Disclosure of Interest: P. Turecek Employee of: Baxter Innovations GmbH, H. Gritsch Employee of: Baxter Innovations GmbH, C. Apostol

tol Employee of: Baxter Innovations GmbH, S. Romeder-Finger Employee of: Baxter Innovations GmbH, A. Bauer Employee of: Baxter Innovations GmbH, D. Burger Employee of: Quintiles, A. Hunt Employee of: Baxter Healthcare Corp.

PO230-WED

Significance of endogenous von Willebrand factor in development of factor VIII immune responses in severe experimental hemophilia A

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Background: A major complication in treating hemophilia A patients is the development of anti-factor VIII (FVIII) immune responses. Several factors have been associated with inhibitor development, including complex physiological or genetic factors. One contentious factor relates to the protective role of von Willebrand factor (VWF) and the plasma-derived or recombinant origin of the FVIII products.

Aims: To evaluate the importance of endogenous VWF in FVIII immunogenicity.

Methods: Wild-type FVIII (FVIII^{HSQ}) and FVIII^{Y1680C} mutant was expressed and purified as previously described. *In vitro* uptake assays were performed using monocyte-derived dendritic cells (MODCs) from healthy donors. FVIII clearance and *in vivo* immunization were performed in both FVIII-knockout (KO) and FVIII/VWF-KO mice. Immunization was performed upon weekly intravenous administration of 1 µg purified FVIII or FVIII mutants in both KO strains. Anti-FVIII antibody titers were measured using ELISA or inhibitor assays.

Results: Specific activities of FVIII^{HSQ} and FVIII^{Y1680C} were similar (7300 ± 165 and 6300 ± 185 IU mg⁻¹ respectively). FVIII^{Y1680C} exhibited rapid clearance in FVIII-KO mice with a *t*_{1/2} of 20 min similar to that of FVIII^{HSQ} in FVIII/VWF-KO. *In vitro* uptake of FVIII^{Y1680C} by MODCs was comparable to that of FVIII^{HSQ}. However, the magnitude of immune responses to FVIII^{Y1680C} or FVIII^{HSQ} was identical in FVIII-KO mice. The response to FVIII^{HSQ} was similar in both FVIII-KO and FVIII/VWF-KO mice.

Conclusion: An immune response to FVIII initiates rapidly when not in complex with endogenous VWF and thus independent of its circulating half-life. FVIII ability to interact with endogenous VWF is not sufficient to inhibit the development of FVIII immune responses in mouse model of hemophilia A. Therefore we speculate that immune recognition of FVIII or FVIII/VWF complex may involve different mechanisms or cellular types.

Disclosure of Interest: None Declared.

PO231-WED

Comparison of methods for the dosage of FVIII in a new haemophilia center care

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Background: Factor VIII can be measured by a one stage coagulometric assay (COAG) or by a chromogenic assay (CHRO). The latter is based on Factor Xa activity generated in a purified system. Both methods are recommended to study patients with haemophilia.

Aims: to compare COAG and CHRO methods in patients with Haemophilia A (HA) and von Willebrand disease (VWD)

Methods: 63 samples from 33 patients (30 presented HA and three with VWD) were studied. 24 from HA patients were severe, three moderate and three mild HA. Samples from 18 patients were delivered pre and post infusion factor concentrates. CLSI protocols (EP 15-A2) were applied. FVIII was measured by COAG and CHRO automated methods (STA compact STAGO, ACL TOP 300; reagents Stago, IL and Coamatic FVIII). low or normal range calibration curves were used. Samples with results below detection limit of CHRO method were retested using a sample dilution 1/30 with 30 min substrate incubation. In addition 35 post infusion samples were analyzed according the administrated medication (20 ADVATE (AD); 7 HEMOFIL-M (HE); 8 KOATE-DVI (KO)). Results were analyzed with infoStat statistic program.

Results: comparison of 63 samples showed *r* Pearson of 0.98 with a *K* of 0.98 (analytical range of FVIII of 0,1-215%). There were no statistical significant differences between methods (paired *T* test *P* 0.7376). The ratio chrom/coag post medications was: 1.17 for AD; 1.02 for HE and 0.93 for KO. Independent *T* testing showed: AD vs HE *P* = 0.0005; AD vs KO *P* < 0.0001; HE vs KO *P* = 0.0646

Conclusion: There was a good correlation between methods along the analytical range. However in samples from patients post infusion of factor VIII concentrate, the ratios chrom/coag were different depending on the type of concentrate administered. This could be due to differences in the factor VIII molecules (B domain) in recombinant products. In patients receiving them, factor VIII measurement by using specific standards of the product as calibrators should be considered.

Disclosure of Interest: None Declared.

PO232-WED

Critical quality attributes of recombinant FVIII products – protein aggregates and subvisible particles

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Background: Neutralizing antibodies against FVIII are the major complication in current therapy for hemophilia A patients. Despite progress in explaining the initiation of unwanted immune responses, the root cause for the development of these antibodies remains unclear. Studies with other biotherapeutics (e.g. human interferons, human growth hormone, monoclonal antibodies) have shown that soluble protein aggregates (SPAs) and subvisible particles (SVPs) have an increased immunogenicity potential. Only limited information is available for FVIII products.

Aims: To assess the presence of SPAs and SVPs in recombinant FVIII (rFVIII) products.

Methods: At least three lots of five commercially available rFVIII products were tested for SPAs and SVPs using size exclusion chromatography, micro-flow imaging (MFI) and a novel flow-cytometry based technology. The latter uses a combination of size calibration beads, counting beads and different fluorescent probes for distinct structural elements to facilitate the assessment of amount, size and nature of SVPs.

Results: SPAs and SVPs were found in all freshly reconstituted rFVIII products. SPA concentrations ranged from 0.6% in one of the full-length (FL-) rFVIII products to 8.4% in one of the B-domain deleted (BDD-) rFVIII products. SVP concentrations varied between 0.7×10^6 SVPs per 1000 IU in one of the FL-rFVIII products to 114.0×10^6 SVPs per 1000 IU in one of the BDD-rFVIII products. There were differences between FL-rFVIII products and BDD-rFVIII products and between individual FL rFVIII products. Most SVPs were protein particles; their structural properties were similar in all freshly reconstituted products tested.

Conclusion: We show that freshly reconstituted rFVIII products contain both SPAs and SVPs, albeit at varying concentrations. It remains

to be shown if and how these aggregates and subvisible particles contribute to the initiation of unwanted immune responses against rFVIII

Disclosure of Interest: M. Malisaukas Employee of: Baxter Innovations GmbH, C. Lubich Employee of: Baxter Innovations GmbH, T. Prenninger Employee of: Baxter Innovations GmbH, P. Matthiessen Employee of: Baxter Innovations GmbH, P. Turecek Employee of: Baxter Innovations GmbH, F. Scheiflinger Employee of: Baxter Innovations GmbH, B. Reipert Employee of: Baxter Innovations GmbH.

PO233-WED

FVIII OS/CS ratios for select novel recombinant fviii replacement products measured in four FDA approved FVIII one-stage clot assay systems and one chromogenic assay

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Background: Reagent dependent over- and/or under-recoveries have been described for several of the novel rFVIII replacement products in the one-stage (OS) clot assay but there is limited published data using a chromogenic (CS) assay.

Aims: One-stage clot FVIII activity was determined for select novel full length (FL) and B-domain deleted (BDD) rFVIII replacement products using four of the most commonly used FDA-approved OS assay systems in the US. In addition, FVIII activity was measured using a single, RUO, chromogenic assay. FVIII OS/CS ratios for novel rFVIII products were compared to OS/CS ratios obtained for ADVATE® (Baxter International) and ReFacto® AF (Pfizer).

Methods: FVIII OS and CS recoveries were determined for five rFVIII products spiked at 80% (based on labeled potency) into hemophilia A plasma (HRF, Inc.). FVIII OS activities were measured using four FDA approved assay systems following manufacturer recommended protocols, calibration and QC: STA-R Evolution® using PTT A and C.K. Prest® (Diagnostics Stago Inc.), BCS® XP using Dade® Actin® FSL (Siemens Healthcare) and ACL TOP® using HemosIL® SynthA-Sil® (Instrumentation Laboratory). FVIII CS activity was measured using manufacturer recommended setup for BIOPHEN FVIII:C on STA-R Evolution®. FVIII OS/CS ratios were determined for all rFVIII products tested.

Results: Recovery of novel rFVIII products was variable in the BIOPHEN FVIII:C CS assay and not all products recovered within $\pm 25\%$ of expected concentration. The OS/CS ratios for BDD-ReFacto® AF fell within 0.8 and 1.2 for three of the four OS assay systems. OS/CS ratios for FL-ADVATE® fell within 0.8 and 1.2 across all OS assay systems. The OS/CS ratios for the two novel BDD-rFVIII products fell within 0.8 and 1.2 in at least two of the OS assay systems tested.

Conclusion: Further evaluation of the behavior of novel rFVIII products in existing FVIII CS assay systems is required.

Disclosure of Interest: None Declared.

PO234-WED

Plasma derived FVIII (PDFVIII) concentrates enhance synovial cell proliferation and migration by binding of misfolded proteins to ADAM15

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Background: Proliferation and inflammation of the synovium are hallmarks of joint damage in hemophilia. ADAM15 triggers inflamma-

tion. We recently discovered that ADAM15 is a pattern recognition receptor for misfolded proteins. Protein misfolding and subsequent protein aggregate formation is a prevalent problem of therapeutic proteins.

Aims: As we observed in the past significantly enhanced synovial cell proliferation in the presence of pdFVIII, but not of rFVIII, we studied now whether this effect is linked to ADAM15.

Methods: The effect of three different recombinant FVIII (rFVIII) products (Kogenate®Bayer, Advate® Baxter, ReFacto AF® Pfizer) and two selected pdFVIII products (Octanate® Octapharma, Haemoclin SDH® Biotest) was investigated. Primary synovial cells were cultured and up to 1 IU mL⁻¹ factor VIII concentrate were added. Binding of protein from FVIII concentrates to a peptide from ADAM15 (ADAM15P) was measured by ELISA and by plasmon resonance. Misfolded proteins and protein aggregates were removed from FVIII concentrates by affinity adsorption on immobilised ADAM15P. Synovial tissue from surgery was got from a hemophilia A patient with informed consent. Protein aggregates were analysed by Nanoparticle tracking analysis.

Results: A specific antibody to ADAM15 showed strong staining of synovial tissue from a hemophilia A patient. Many different proteins were found inside the pdFVIII products that bound to the 'misfolding probe'ADAM15P peptide. We verified that the used pdFVIII products, in contrast to the rFVIII products, strongly enhanced synovio-cyte proliferation and migration. But after removal of misfolded proteins by affinity adsorption on ADAM15P, the increase in synovio-cyte proliferation was completely gone. Affinity adsorption removed ADAM15P binding proteins and strongly reduced protein aggregates, but did not reduce FVIII activity.

Conclusion: The enhanced synovial cell proliferation and migration induced by pdFVIII is likely mediated by ADAM15.

Disclosure of Interest: None Declared.

PO235-WED

Are different plasma derived FVIII concentrates equally efficient in tertiary prophylaxis?

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Background: Patients in Serbia are usually switching between different plasma derived FVIII concentrates (pdFVIII), dependent on their market availability. We observed that some of the patients developed a personal impression that one factor concentrate is more efficient in bleeding control than others.

Aims: To investigate whether five different pdFVIII (Immunate, Baxter; Beriate P, CSL Behring; Koate HP, Bayer Schering Pharma; Octanate, Octapharma AG; Haemoclin, Biotest Pharma) are equally efficient in joint hemorrhage prevention in adult patients on tertiary prophylactic regimen.

Methods: Eight adult patients, average age of 29, with severe form of hemophilia A, received a low dosage tertiary prophylaxis. Over a 4-year observation period from January 2010 to March 2014, our patients received, twice a week, the same dosage (15U/kg) of pdFVIII. During this time they switched between different pdFVIII, dependent on the availability at that specific point of time. Repeat inhibitor testing in all eight patients was negative. We extracted from a personal patient diary the data about type of pdFVIII they received and the incidence of joint bleeding episodes, and calculated average monthly bleeding rate for every pdFVIII concentrate

Results: Eight patients received altogether Immunate during 88 months, Beriate P 83 months, Koate HP 68, Octanate 79 and Haemoclin during 82 months. For Immunate average bleeding frequency per month was 0.53, for Beriate P 0.52, Koate HP 0.52, Octanate 0.59, Haemoclin 0.58. During the observed period average bleeding rate per month per patient was 0.90, 0.28, 0.42, 1.02, 0.84, 0.68, 0.24, and 0.12, respectively. There was not a statistically significant difference in spon-

taneous bleeding occurrence while using different pdFVIII as determined by one-way ANOVA ($F(2,27) = 0.140$, $p = 0.996$).

Conclusion: According to our results, all five investigated pdFVIII are equally efficient in joint bleeding prevention in adult patients that are on tertiary prophylactic regimen

Disclosure of Interest: None Declared.

Fibrinogen / fibrin / D-dimer III

PO236-WED

Changes of coagulation parameters during high altitude expedition

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Background: Both coagulation and fibrinolytic activity are involved in the genesis of high altitude pulmonary edema. Many studies were performed at altitudes higher than 5000 masl, but only a few were done at lower altitudes. D Dimmer (DD) is a very sensitive marker of coagulation activation

Aims: To measure DD and other hematological and hemostatics parameters in a group of climbers (13 subjects, six women and seven men) on a 3-day expedition in Puente del Inca, Mendoza, Argentina, reaching 3500 masl and sleeping at 2800 masl.

Methods: Blood tests were done before and immediately after climbing, considering those conditions that could change the samples quality. DD (chemiluminescence), automatic cell blood count, C-reactive protein (CRP) (immunonephelometric), and fibrinogen (Clauss Method) were measured. Male's results were compared to female's and represented as mean values and standard deviation. Comparison between the two groups was done using the MannWhitney U-test.

Results: The climbers did not show any symptoms of acute mountain sickness (AMS). There was a significant elevation of DD (pre: 219.8–136.03 ng mL⁻¹; range: 82.4– 613.5 ng mL⁻¹; post: 609.89–918.57 ng mL⁻¹; range: 203.9– 3586.8 ng mL⁻¹; $P < 0.01$). There were no changes in platelet count, CRP, or fibrinogen. Highest values of DD were observed in women.

Conclusion: The elevation of DD concentration may show that there was coagulation activity at moderate altitudes in a group of Argentinian mountaineers without symptoms of AMS. Ingestion of oral contraceptive and/or hormonal changes may be reasons of higher values of DD in women.

Disclosure of Interest: None Declared.

PO237-WED

Relationship between oxidative stress and blood coagulation in paediatric chronic kidney disease (CKD)

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Background: CKD is characterized by progressive renal insufficiency, accompanied by uremic toxins accumulation, in addition to an imbalance between antioxidant pro-oxidants substances, which results in oxidative stress. This condition has been linked to inflammation, which in turn is associated with blood coagulation activation. CKD progression is complicated by complex hemostatic changes, expressed clinically by either bleeding or thrombotic events.

Aims: This study aims to investigate the relationship between oxidative stress and blood coagulation in CKD pediatric patients caused by glomerular lesions, CAKUT and other etiologies.

Methods: This study was approved by the local Ethical Committee and consent was obtained in all cases. It includes 54 pediatric CKD patients (6–18 years old) distributed in Group I-CKD caused by glomerular diseases ($N = 20$), which was divided into Subgroup IA- stages 1 or 2 ($N = 11$) and Subgroup IB- stages 3 or 4 ($N = 9$); Group II-CKD stages 3 and 4 caused by CAKUT ($N = 26$), Group III- CKD in stages 3 or 4 by other etiologies ($N = 8$) and Group IV(II+III), $N = 43$. A control group-V included 52 healthy children, sex and age matching. Plasma levels of thiobarbituric reactive species acid (TBARS), proteins carbonyl (Protein Carbonyl Assay-Cayman), anti-oxidant plasma (MTT reduction) and ascorbic acid (Ferric reducing ascorbate-BioVision) were determined. D-dimer levels (Seikisui) were determined as a marker for coagulation.

Results: The proteins carbonyl levels were lower in group I and subgroup IB vs control ($P = 0.00$ in both cases). D-Di levels were lower in subgroup IA vs groups II ($P = 0.02$) and IV ($P = 0.03$) and higher in groups II and IV vs control ($P = 0.00$ in both cases). Only few poor correlations were obtained among data.

Conclusion: D-Di levels suggest higher blood coagulation in CKD severe stages. However there was not a relationship between oxidative stress and coagulation in CKD. Interference in oxidative stress markers evaluation *in vitro* should not be ruled out. Support: CNPq and FAPEMIG/Brazil.

Disclosure of Interest: None Declared.

PO238-WED

Influence of oxidative stress onto fibrin net formation, architecture and lysis

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Background: Fibrin network formation is an important part of hemostasis. Modification of fibrinogen by products of oxidative stress changes its chemical and physical properties due to cross-linking, partial fragmentation, aggregation and new groups forming.

Aims: The aim of this study was to observe influence of fibrinogen modification onto formation, architecture and lysis of fibrin net.

Methods: Three different reagents and their combinations were selected for fibrinogen modification. Malondialdehyde (MDA), a product of lipid peroxidation; sodium hypochlorite (NaOCl), a product of immune responses; 3- morpholinosydnnonimine (SIN-1), represents reactive nitrogen species. For determination of degree of fibrinogen modification carbonyl groups evaluation was used. Fibrin net formation and lysis were measured by turbidimetric method. Fibrin net architecture was studied by confocal and scanning electron microscopy. For identification of modified amino acids residue mass spectroscopy was used.

Results: New carbonyl groups in fibrinogen were formed by all species and its combinations. The highest count of carbonyls was obtained by combination MDA-NaOCl. Formation and lysis of fibrin nets produced from modified fibrinogens were affected by all modification systems in comparison with controls. Fibrin net architecture from modified fibrinogen was altered when compared to control. NaOCl influence overrode MDA or SIN-1 effect. Modified fibrinogen amino acids residues were found in sequences important for formation and lysis of fibrin net.

Conclusion: Better understanding of fibrinogen modification and its influence on fibrin net formation and architecture might help in evaluation of thrombotic/bleeding complications linked with oxidative stress in diseases.

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Disclosure of Interest: None Declared.

PO239-WED

CD44 located in the surface of sickle red blood cell (SRBC) correlates with reduced blood HbF levels and increased SRBC adhesion to fibrin

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Background: Adhesion molecules such as CD44 are present in the surface of sickle red blood cells (SRBC). They are more active binding to other circulating cells and/or to vessels wall during particular haemostatic conditions as sickle cell disorders (SCD). CD44 is known to interact with hyaluronan and is believed to bind to fibronectin and fibrin. However, the SRBC CD44 involvement with SCD remains elusive.

Aims: In this study, hemolysis, reticulocytes, total plasma (Hb) and fetal hemoglobin (HbF) levels in the blood collected from 12 patients with SCD HbSS (n = 10) or with HbSC (n = 2) during steady state were estimated. The results were correlated with the presence of CD44 in the RBC membrane and adhesion capacity of SRBC.

Methods: Basal adhesion of RBC to fibrin(ogen) and matrix gel was measured using static adhesion assays and flow cytometric detection of surface protein expression.

Results: The levels of CD44 in RBC negatively correlated ($r = -0.6702$, $P = 0.017$) with the levels of HbF. No correlation was found among lactate dehydrogenase, reticulocyte count and total hemoglobin and CD44 levels. In terms of adhesion, CD44 levels positively correlated ($r = 0.771$, $P = 0.003$) with the adhesion of RBC to fibrin. No correlation was found among RBC adhesion to fibrinogen or matrix gel and the levels of CD44.

Conclusion: These results suggest that blood coagulation depends on SRBC CD44 binding to fibrin in the vessels wall. Therefore, the observed negative correlation between HbF and CD44 abundances links higher levels of HbF with a better prognosis for vessel occlusive events susceptible patients.

Disclosure of Interest: None Declared.

PO240-WED

Free fatty acids modulate thrombin activity on fibrinogen and destabilise fibrin clots

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Background: The effectiveness of fibrinolysis depends on the catalytic efficiency of the protease and the structure of the fibrin substrate. The modulatory effect of free fatty acids (FFAs) on the activity of plasmin, the primary fibrinolytic protease is well known, but the FFA effects on thrombin activity, which profoundly influences the fibrin structure, are less characterized.

Aims: To investigate the polymerization kinetics, structure and mechanical stability of fibrin generated from purified fibrinogen by thrombin in the presence of FFAs.

Methods: The fibrinogen-fibrin conversion was followed with turbidimetry in the presence of sodium salts of oleic and stearic acid. Viscoelastic properties of the clots were measured with rotatory oscillation rheometer. Scanning electron microscopy (SEM) was used to detect the changes in morphology of fibrin meshwork. Autodigestion of thrombin in the presence of FFAs was examined by SDS PAGE to explore direct molecular interactions.

Results: In turbidimetric assays oleate and stearate produced a bell-shaped inhibitory dose response of fibrin generation: the time needed to reach half-maximal turbidity was increased 10–30 fold at the most effective modulator concentrations (100 μ M oleate, 300 μ M stearate). Storage (G') and loss (G'') moduli of fibrin decreased from $G' = 73$ and $G'' = 7.9$ Pa to $G' = 36$ and $G'' = 3.9$ Pa at 100 μ M oleate and to $G' = 46$ and $G'' = 5.4$ Pa at 100 μ M stearate. Morphometric analysis of SEM images showed significant changes in fiber size, median diameter increased from 50.5 to 87.3 nm in the case of stearate and decreased from 62.0 to 49.4 nm in the case of oleate. Autodigestion of thrombin was accelerated by both modulators but at different rates and with different size profile of the degradation products.

Conclusion: Oleate and stearate at concentrations in the submillimolar range alter thrombin mediated fibrin generation resulting in less stable clots.

Disclosure of Interest: None Declared.

PO241-WED

Temporal evolution of fibrin nanostructure during clotting and fibrinolysis

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Background: Analysis of visible light spectra transmitted through forming and lysing fibrin clots yields the temporal evolution of its nanostructure, e.g. the protofibrils number per fiber (1). This parameter has a major functional role in clot stability and resistance to fibrinolysis.

Aims: Quantify the differences in the temporal evolution of the protofibrils number during fibrin polymerization, maturation and lysis in normo-, hypo-, and hyper-coagulant or fibrinolytic plasmas.

Methods: Frozen human plasmas either immuno-depleted or from patients, were characterized by using routine methods, Thrombin Generation and factor determinations including TFPI and PAI. The plasmas incubated with low Tissue Factor concentration +/- t-PA were triggered by calcium. The number of protofibrils was measured in real time during 30 min at 37°C on a prototype routine instrument (1).

Results: Different temporal evolutions of the clot nanostructure were obtained during fibrin formation, maturation and fibrinolysis, in agreement with the plasmas expected hemorrhagic or thrombotic profiles. Experimental conditions and procedures were tuned to optimize the discrimination between different plasma profiles.

Reproducibility of the measurement is excellent with CVs <5% for coagulation and <7% for lysis, whatever the plasmas. Identical results (within error bars) were obtained from fresh and frozen plasma, allowing evaluation of patients' plasma collections.

Conclusion: The temporal evolution of fibrin clots nanostructure during fibrin polymerization, maturation and lysis outlined the expected differences between control plasmas. In particular a quantitative discrimination between retarded or accelerated formation and/or lysis is obtained. This automated method opens new perspectives in the management of bleeding and thrombotic risk, as well as individual stratification of patients. (1) Yeromonahos et al. Biophysical J. 2010, Arterioscler Thromb Vasc Bio. 2012.

Disclosure of Interest: None Declared.

PO242-WED

Fibrinogen levels during the last stage of pregnancy in Mexican women

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Background: Peripartum hemorrhage (PH) is a public health problem worldwide and in Mexico it is a leading cause of maternal death. A procoagulant state has been described during pregnancy however abnormal fibrinogen (Fg) levels or a hyperfibrinolytic state with Fg consumption may lead to hemorrhagic events.

Aims: To assay Fg levels and fibrinolytic activity during the last month of pregnancy in Mexican women.

Methods: We evaluated 100 women ≥ 37 weeks of gestation. Fifty had normal pregnancy (NP) and 50 had preeclampsia (PE). A control group (non-pregnant women; $n = 50$) was included. All women gave their informed consent. We assayed antigenic (AgFg, ELISA) and functional Fg (Fg, Clauss), PAI-1, D-dimer (D-D), fibrinogen-fibrin degradation products (FDPs), thrombin time (TT), and euglobulin lysis time (ELT). The study was performed according to the Declaration of Helsinki and it was approved by the Ethical Committee of our Institution.

Results: None women had prolonged TT. Fg levels were higher ($P \leq 0.05$) in women with PE (AgFg=374.3 and Fg = 389.2 mg dL⁻¹) than in women with NP (AgFg = 292.5 and Fg = 309.6 mg dL⁻¹) and controls (AgFg = 305.2 and Fg = 316 mg dL⁻¹). No significant difference in Fg levels were found between NP and control groups (≥ 0.05). PAI-1 levels were 53.4 and 55.1 ng mL⁻¹ for NP and PE groups, respectively. These levels were significantly higher than in the control group (PAI-1 = 40 ng mL⁻¹). D-D and FDPs were positive ($\geq 0.5 \mu\text{g dL}^{-1}$) for NP and PE groups. Finally, ELT was always normal in all groups (≥ 120 min).

Conclusion: Results show an activated fibrinolytic system in pregnant women but not a hyperfibrinolytic state since ELT results were negative. Increase in PAI-1 and Fg levels suggest a procoagulant state, mainly in the PE group. Interestingly, Fg levels in women with NP did not increase as expected. These findings should be studied further in order to establish whether unchanged Fg levels during the last stage of pregnancy contribute to the development of PH.

Disclosure of Interest: None Declared.

PO243-WED

Understanding fibrin protofibrils at the molecular level: formation and flexibility through a new coarse-grained protein modelling approach

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Background: The process of fibrin clot formation, together with the mechanical properties that arise from its topology are subjects of great bio-medical interest. Despite of recent experimental advances several limitations to understand such structures at the molecular level still remain. Removing these barriers in our knowledge is essential if we are to fully understand clot formation and the diseases associated with this.

Aims: Computer simulations using atomistic molecular dynamics are well established as a theoretical tool for structural characterisation to complement experimental studies, but the high computational expense

of the calculations means that simulations of fibrin aggregation are unfeasible, even with modern supercomputers. We aim to overcome this limitation using novel approaches.

Methods: To overcome this limitation, we have developed a new coarse-grained model for proteins known as FFEA (Finite Element Molecular Analysis) which treats proteins as a non-rigid continuum subjected to thermal fluctuations. In this work, we adjusted the Young's modulus, which defines molecular flexibility within FFEA, for the fibrin monomer to reproduce negative stain electron microscopy data.

Results: By using this novel approach we have simulated the dynamics of a half-staggered protofibril, thus unveiling its flexibility patterns. In addition, we explored the interaction space for the A:a and B:b knob-hole as well as the D-D (end-to-end) interaction sites giving rise to the first events in protofibril formation.

Conclusion: This novel FFEA approach can ultimately be applied to the rational design of a new generation of drugs that modulate the interaction strengths of fibrin to address the bleeding and thrombotic complications of several diseases.

Disclosure of Interest: None Declared.

PO244-WED

Impact of maternal D-dimer levels in newborn of preeclamptic mothers

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Background: Preeclampsia (PE) is a multi-system disorder of pregnancy by characterized by high blood pressure ($\geq 140 \times 90$ mmHg) and proteinuria (≥ 0.3 g (24 h)⁻¹). The severe form (sPE) is associated with these levels even higher. PE is associated with a prothrombotic state, which promotes fibrin deposition in placenta microcirculation and compromises the perfusion of the fetus. D-dimer (D-Di) is the smallest fragment of the breakdown of fibrin and is considered a marker of coagulation activation.

Aims: To evaluate the impact of maternal D-Di levels in newborn of sPE mothers.

Methods: The local Ethical Committee approved this study and consent was obtained in all cases. A total of 77 newborn medical records of 74 Brazilian sPE women, who had participated in previous studies of our research group, were reviewed. sPE was defined by blood pressure $\geq 160 \times 110$ mmHg and proteinuria ≥ 2 g (24 h)⁻¹. D-Di levels were determined by ELISA (American Diagnostica®).

Results: Data from newborn of sPE women were included. A positive correlation (Spearman) was found between maternal D-Di and the following newborn parameters: weight ($P = 0.002$, $r = 0.341$), middle cerebral artery dilatation ($P = 0.036$, $r = 0.259$), increased resistance in the umbilical artery ($P = 0.006$, $r = 0.308$), presence of oligohydramnios ($P = 0.042$, $r = 0.233$), days of hospitalization ($P = 0.009$, $r = 0.297$), and days of intensive therapy ($P = 0.006$, $r = 0.308$).

Conclusion: Our data suggest that the maternal hypercoagulability is associated with worse fetal perfusion by fibrin deposition in placental microcirculation, impairing intrauterine blood supply, which in turn result in worse newborn condition. In this way, D-Di plasma levels can be a useful marker for unfavorable outcomes in newborn of preeclamptic mothers. Support: CNPq, CAPES and FAPEMIG/ Brazil.

Disclosure of Interest: None Declared.

PO245-WED

Fibrin clot structure is affected by levels of particulate air pollution exposure in patients with venous thrombosisPan X^{1,2}, Gong Y³, Martinelli I⁴, Mannucci P⁵, Routledge M¹ and Ariens R²

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Background: Particulate air pollution is a risk factor for cardiovascular complications and thrombosis. Long-term exposure to particulate matter with a diameter < 10 µm (PM₁₀) has been associated with an increased risk of venous thrombosis.

Aims: The aim of this study was to investigate whether particulate air pollution alters fibrin clot structure and thus modulates thrombosis risk.

Methods: We investigated fibrin polymerisation by turbidity, clot structure by confocal microscopy and fibrin pore size by permeability in 103 patients with deep vein thrombosis and 121 healthy controls, for whom levels of air pollution exposure had been recorded. Exposure groups were defined by mean PM₁₀ concentrations over the 730 days before the event.

Results: We found a higher average number of fibres per clot area in the patients than controls, but no difference in K_s or fibre thickness. When the two groups were divided into high or low exposure to PM₁₀, a significantly denser fibrin clot network structure with thicker fibres (higher maximum absorbance, $P < 0.05$), decreased permeability (lower K_s value, $P < 0.05$) and higher average fibre numbers per clot area ($P < 0.05$) was observed in patients in the high exposure group compared to those with low exposure. There were no significant differences in fibrin clot structure between the two exposure levels in healthy subjects.

Conclusion: PM₁₀ levels are associated with altered fibrin clot structure in patients with deep vein thrombosis but not in controls, suggesting that air pollution may trigger differences in fibrin clot structure only in patients predisposed to thrombotic disease. The mechanism for this difference remains to be elucidated.

Disclosure of Interest: None Declared.

PO246-WED

Increased plasma clot permeability and susceptibility to lysis are associated with heavy menstrual bleeding of unknown causeSzczepaniak P¹, Ząbczyk M² and Undas A²

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Background: Formation of compact and poorly lysable clots has been reported in thromboembolic disorders. Little is known about clot properties in bleeding disorders.

Aims: We hypothesized that more permeable and lysis-sensitive fibrin clots can be detected in women with heavy menstrual bleeding (HMB).

Methods: We studied 52 women with HMB of unknown cause and 52 age-matched control women. Plasma fibrin clot permeability (K_s), tur-

bidity and efficiency of fibrinolysis, together with coagulation factors, fibrinolysis proteins, and platelet aggregation were measured.

Results: Women with HMB formed looser plasma fibrin clots (+16% [95%CI 7–18%] K_s) that displayed lower maximum absorbance (-7% [95%CI -9 – -1%] ΔAbs_{max}), lower maximum D-dimer levels released during proteolysis (-7% [95%CI -8 – -1%] D-D_{max}), and shorter clot lysis time (-17% [95%CI -23 – -11%] CLT). The HMB patients and controls did not differ with regard to coagulation factors, fibrinogen, von Willebrand antigen, thrombin generation markers and the proportion of subjects with defective platelet aggregation. The patients had lower platelet count (-12 [95%CI -19 – -2%]), tissue plasminogen activator antigen (-39% [95%CI -41 – -29%] tPA:Ag), and plasminogen activator inhibitor-1 antigen (-28% [95%CI -38 to -18%] PAI-1:Ag) compared with the controls. Multiple regression analysis upon adjustment for age, body mass index, glucose, and fibrinogen showed that decreased tPA:Ag and shortened CLT were the independent predictors of HMB.

Conclusion: Increased clot permeability and susceptibility to fibrinolysis are associated with HMB, suggesting that altered plasma fibrin clot properties might contribute to bleeding disorders of unknown origin.

Disclosure of Interest: None Declared.

PO247-WED

Severe thrombosis in pregnancy in patient with congenital dysfibrinogenemia

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Background: In a 26-year-old woman, diagnosis of congenital dysfibrinogenemia was made due to combination of family and personal history of recurrent pulmonary embolism and abnormalities of thrombin time and reptilase time in otherwise normal coagulation screening tests panel (level of fibrinogen was 2.59 g L⁻¹). The patient had no comorbidities. Screening for hereditary thrombophilia was negative. Mutation in her fibrinogen gamma chain (gamma 363Tyr/Asn) was found and published as a novel mutation in 2009.

Aims: The patient presented with dyspnoea and pleuritic chest pain in week 7 of her pregnancy while on prophylactic anticoagulation therapy with LMWH.

Methods: Her D-dimer was strongly elevated. Nevertheless, the pulmonary embolism was excluded at the emergency department. Because of persistence of clinical symptoms and D-dimer elevation, therapeutic dose of LMWH was given by hematologist. Three weeks later, the patient was admitted to the hospital with severe shortness of breath and exhaustion.

Results: The diagnosis of submassive pulmonary embolism was confirmed and high therapeutic doses of LMWH were administered. An inferior vena cava filter was inserted in order to prevent future PE recurrence. By several days, the symptoms resolved and the patient was discharged home. Her pregnancy was progressing well according to first trimester fetal ultrasound. She was then maintained on therapeutic doses of LMWH throughout the rest of pregnancy. No more thrombotic events occurred. After the delivery, the LMWH was changed back to long-term warfarin therapy. The vena cava filter was removed 15 months after delivery.

Conclusion: We conclude that although dysfibrinogenemia associated with thrombosis is a very rare condition, it can lead to serious thrombotic complications in pregnancy despite anticoagulation prophylaxis. These can be even life-threatening to mother and fetus when missed.

Disclosure of Interest: None Declared.

Hemophilia clinical III

PO248-WED

Arteriovenous fistulas for replacement therapy in adults with hemophilia

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Background: Intravenous self-infusion of clotting factor concentrates is difficult in adult hemophilia patients with poorly accessible peripheral veins or with significant elbow arthropathy. In such cases arteriovenous fistulas (AVF) are attractive alternatives to central venous devices, in terms of requirements for care, ease of access, and cosmetic appearance. There have been two reports of successful creation and use of AVF in large pediatric hemophilia clinics, together totaling 53 AVF procedures in 47 patients, all but one of whom were aged ≤ 13 (McCarthy et al 2007; Mancuso et al, 2009).

Aims: To assess AVF for home self-infusion in adults with hemophilia in whom peripheral IV access is impractical.

Methods: Anatomy was suitable for AVF creation in five of eight potential candidates. Two were on prophylactic infusion schedules and three were treated on-demand. Their ages were 18–72 at the time of AVF creation (median 40). Three have moderately severe hemophilia A (HA), one has severe hemophilia B (HB), one has type 3 VWD. In one patient the AVF had been created elsewhere in 1995 for hemodialysis, and has been used for factor VIII infusions since he received a renal allograft. The other four AVF were created in our institution between November 2013 and May 2014, three of them by the same vascular surgeon. Three AVF were radio-cephalic and one was ulnar-cephalic.

Results: There was no major surgical blood loss or other complications; local edema was noted in one patient. Clotting factor usage perioperatively (4–6 days) was 152–335 IU kg⁻¹ factor VIII, IX, or VWF/VIII concentrate. All AVF matured and are being accessed for home self-infusion. One patient required balloon angioplasty at 6 months for stenosis at the proximal venous outflow tract. One patient remains on an on-demand treatment schedule; the other four are now on prophylactic schedules, either alternate day or twice weekly (the HB patient).

Conclusion: AVF can facilitate venous access for home management of hemophilia in adults who have difficulty accessing native veins.

Disclosure of Interest: None Declared.

PO249-WED

A phase III, open-label, multicentre study to evaluate efficacy and safety of a plasma-derived von Willebrand factor/factor VIII concentrate in pediatric subjects with hemophilia A (Swiftly-HA study)

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Background: Voncento® comes with a low-volume and the solution contains a VWF:FVIII ratio of 2.4:1. The SWIFT ('Studies with von

Willebrand factor/Factor VIII') program is evaluating this product in hemophilia A and VWD patients in accordance with the European clinical and pediatric guidelines.

Aims: This was a Phase III study to investigate the efficacy and safety in pediatric subjects aged 0 to <12 years with severe hemophilia A (FVIII:C < 1%) who had received previous FVIII treatment for a minimum of 20 exposure days. The pharmacokinetic data are presented in abstract ISTH15ABS-1374.

Methods: Thirtyfive subjects received Voncento® as on-demand therapy (n = 17: 12 were <6 years including four subjects < 2 years) or as prophylactic therapy (18 subjects: four were < 6 years). All subjects were analyzed for hemostatic efficacy and safety; Subjects were treated up to 100 exposure days with a maximum study duration of 12 months.

Results: The 17 on-demand subjects reported 320 non-surgical bleeds (NSB) and received a median number of 29.0 infusions (median dose of 34.2 IU FVIII kg⁻¹). The hemostatic efficacy was assessed by the investigator as excellent/good in all cases (24%/76%). The 18 subjects in the prophylaxis arm (10 were previously naive to prophylaxis) experienced 173 NSB events (97 NSBs (56%) were reported by three subjects). Five subjects (28%) had no NSB. All received a median number of 92 infusions (median dose 30.6 IU FVIII kg⁻¹). The majority of the bleeds (92%) needed only one infusion. Hemostatic efficacy was assessed by the investigator as excellent (86%) or as good (14%). Inhibitors occurred in three subjects (2 low-, one high titer) of which 1 disappeared (low titer) and 2 persisted. These three subjects had a median of 22 previous exposure days. No other safety findings of concern were observed.

Conclusion: This comprehensive program evaluating Voncento® across all ages demonstrates excellent safety and efficacy for treatment and prevention of bleeds in children with severe hemophilia A.

Disclosure of Interest: M. T. Pompa: None Declared, G. Iosava: None Declared, C. Khayat: None Declared, I. Romashevskaya: None Declared, O. Aleinikova: None Declared, O. Stasyshyn: None Declared, L. Vashchenko: None Declared, M. J. Lopez: None Declared, W. Seifert Employee of: CSL Behring.

PO250-WED

Joint lavage followed by viscosupplementation and triamcinolone in patients with severe hemophilic arthropathy. Subjective pain, function and quality of life results

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Background: Young patients sequelaed by multiple joint bleeds waiting for the prosthetic treatment can improve function and quality of life of multiple joints with viscosupplementation enabling appropriate rehabilitation and the correct choice of surgical procedure of each joint. Viscosupplementation results can be improved by previous joint lavage and by adding triamcinolone.

Aims: To assess whether joint lavage, viscosupplementation and triamcinolone improves joint pain, function and quality of life in patients with severe hemophilic arthropathy.

Methods: Fourteen patients with knee hemophilic arthritis with and without involvement of other joints underwent joint lavage and subsequent injection of Hyal G-F20 and triamcinolone in all affected joints. The patients answered the algo-functional questionnaires (Lequesne and WOMAC), visual analog scale for pain (VAS) and SF-36 (quality of life) preoperatively, and at one and three, six and twelve months postoperatively.

Results: Sixteen knees, 15 ankles, 8 elbows and one shoulder were treated in 14 patients. Six patients had musculoskeletal bleeding [ankle (1), leg muscle (2) and knees(4)] at 3 months affecting results. Pain (VAS and WOMAC pain) did not show significant improvement ($P = 0.7$, $P = 0.06$, respectively). However function had significant improvement at one month that improved more at one year given by WOMAC (average 10 points, $P = 0.02$). Function defined by the Lequesne questionnaire also improved (from 10.1 to 7.3, in average) but more significantly at one and three months ($P = 0.1$). The physical component of the SF36 presented clinically relevant and significant improvements in all time points except for 3 months follow-up, with best results at one-year follow-up (baseline = 33.4; 1 month = 39.6; 3 months = 37.6; 6 months 92.6 and 1 year = 44.6; $P < 0.001$).

Conclusion: Joint lavage followed by injection of corticosteroids and Hylan G-F20 is effective in the treatment of hemophilic arthropathy, even if severe, improving function and quality of life.

Disclosure of Interest: None Declared.

PO251-WED

Interim results from a large multinational extension trial (Guardian TM2) using turoctocog alfa for prophylaxis and treatment of bleeding in patients with severe haemophilia A

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Background: Turoctocog alfa (NovoEight®), a recombinant factor VIII (rFVIII) product, has been approved by major regulatory authorities for the prophylaxis and treatment of bleeding in patients with haemophilia A (HA). Guardian™2 is a large, multinational, extension trial of turoctocog alfa in HA patients from 19 countries who were previously enrolled in the pivotal guardian™1 and guardian™3 trials.

Aims: The objective of this ongoing trial is to assess the long-term safety and efficacy of turoctocog alfa for the prevention and treatment of bleeds.

Methods: Previously treated patients (age 1–61 years) with severe HA (FVIII activity $\leq 1\%$) without inhibitors, received prophylactic therapy with scheduled preventive turoctocog alfa injections (20–50 IU kg⁻¹ once every second day or 20–60 IU kg⁻¹ three times weekly), with additional treatment for bleeding episodes. Six patients received turoctocog alfa on-demand for bleeds as they occurred.

Results: This interim analysis includes data from > 4 years for the first patient enrolled in the trial, and a total of 451.6 patient-years. As of the cut-off date (31 Dec 2013), the cumulative exposure to turoctocog alfa was 72,320 exposure days (ED); the mean exposure was 361.6 ED/

patient. No FVIII inhibitors (≥ 0.6 BU) were detected and no safety issues were identified. The overall estimated annualised bleeding rate (ABR, Poisson estimate) for patients of prophylactic treatment was 2.78 and the median ABR was 1.56. The overall mean and median joint ABR were 2.10 and 0.93, respectively; non-joint mean and median ABR were 0.67 and 0.35, respectively. The overall success rate for treatment of bleeds during the preventive regimen was 89% and 90% of all bleeds were successfully treated with ≤ 2 injections of turoctocog alfa.

Conclusion: The interim results of the guardian™2 clinical trial show that turoctocog alfa provides long-term safety and efficacy in the prophylaxis and treatment of bleeds in patients with severe HA.

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PO252-WED

Validation of the international society on thrombosis and haemostasis bleeding assessment tool (ISTH-BAT) in hemophilia carriers

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Background: The ISTH-BAT is a standardized, quantitative tool developed to assess bleeding symptoms. It has not yet been studied in hemophilia carriers.

Aims: Our aim is to validate the expert-administered ISTH-BAT as a screening tool in hemophilia carriers for the identification of those with low factor VIII or IX levels. We present preliminary results of this ongoing study.

Methods: This is a multinational, prospective, observational, cross sectional study. After obtaining approval from the Institutional Review Boards at each recruitment site, informed consent was obtained from hemophilia A or B carriers 18 years or older, with documented FVIII

or FIX levels, with or without bleeding symptoms. The ISTH-BAT, translated into several languages by a professional translation team, was administered to all carriers. Psychometric reliability of the ISTH-BAT (test retest and inter-observer reliability) was conducted in a subset of carriers.

Results: To date, 67 carriers have been recruited from five countries, (62 hemophilia A, 5 hemophilia B) with a mean age of 42 years (range 20-82). The most common reported symptoms include bruising, oral cavity bleeding, bleeding after surgery, tooth extraction and delivery, as well as menorrhagia. 28 have positive bleeding scores (median score of 11, range 6-28) of which 16 have low factor levels (median of 0.33 IU mL⁻¹, range 0.17-0.49) and 12 have normal factor levels (range 0.56-1.10 IU mL⁻¹). 39 have negative bleeding scores (median of 2, range 0-5) of which 7 have low factor levels (median of 0.38 IU mL⁻¹, 0.27-0.48); the remaining 32 have normal factor levels (0.55-1.75 IU mL⁻¹). Therefore, the calculated sensitivity is 70%, specificity is 73%, PPV is 0.57, and NPV is 0.82. The ICC for test/retest (same observer) was 0.906 (n = 7) and ICC for test/re-test (inter-observer) was 0.957 (n = 4).

Conclusion: Preliminary results of this ongoing study suggest that the ISTH-BAT may be a useful tool in the evaluation of bleeding symptoms in hemophiliacarriers.

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PO253-WED

Efficacy and safety of recombinant fusion protein linking coagulation factor IX with albumin (RIX-FP) in previously treated patients with hemophilia B undergoing a surgical procedure

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Background: A fusion protein genetically linking recombinant human coagulation FIX with recombinant human albumin (RIX-FP) has been developed with an improved PK profile, improving hemophilia B treatment by allowing less frequent dosing and lower FIX consumption for perioperative management.

Aims: A surgical sub-study was included in 3 phase III studies, including a pediatric study in subjects with hemophilia B, as part of the glo-

bal PROLONG-9FP clinical program. This report focuses on the use of rIX-FP for perioperative prophylaxis.

Methods: Efficacy was evaluated by Investigator assessment (4-point scale), predicted and estimated actual blood loss, and consumption of rIX-FP during the 14-day perioperative period. Safety, including inhibitors to FIX was assessed. The pre-operative dose of rIX-FP was based on individual incremental recovery (target FIX: 80-100%). FIX activity was monitored at local laboratories to determine repeat dosing during the surgical period.

Results: Ten (10) males (ages 8-51 years) with hemophilia B (FIX ≤ 2%) underwent a total of 12 major surgeries, including 5 orthopedic surgeries with rIX-FP. Hemostatic response was rated by investigators as excellent or good for all surgeries. A single dose of rIX-FP before surgery was sufficient to maintain hemostasis during surgery. Estimated actual blood loss was consistent with predicted blood loss in patients without hemophilia B. The total number of rIX-FP injections ranged from 2 to 7 over the 14 day study period for all surgeries. For orthopedic surgeries (n = 5), the median rIX-FP consumption was 87 IU kg⁻¹ pre-operatively (Day 0), 51 IU kg⁻¹ during post-op Day 1-2 (24-72 h), and 340 IU kg⁻¹ overall (Day 0-14), with a range of 6-7 injections. No subjects developed inhibitors to FIX or antibodies to rIX-FP during the study.

Conclusion: These results support the efficacy and safety of rIX-FP for perioperative management in patients with hemophilia B, with prolonged dosing intervals and lower FIX consumption than conventional therapies.

Disclosure of Interest: C. Negrier Consultant for: CSL Behring, L. M. Lepatan: None Declared, A. Lubetsky: None Declared, F. A. Karim: None Declared, B. Pan-Petes: None Declared, A. Tagliaferri: None Declared, C. Altisent: None Declared, J. Curtin Grant/Research Support from: CSL Behring, Consultant for: CSL Behring, Baxter Biosciences, Biogen Iden, Novo Nordisk, Speaker Bureau of: CSL Behring, C. Male Speaker Bureau of: CSL Behring, A. Lienhart: None Declared, D. Wolko Employee of: CSL Behring, C. Voigt Employee of: CSL Behring, I. Jacobs Employee of: CSL Behring, E. Santagostino Grant/Research Support from: Pfizer, Consultant for: CSL Behring, Grifols, Bayer, Baxter, NovoNordisk, Biogen/Idec, Sobi, Roche, Pfizer, Speaker Bureau of: Kedrion, Octapharma, Biotest.

PO254-WED

Comparison of annual bleeding rates in subjects with severe hemophilia A receiving prophylaxis treatment with recombinant factor VIII or pegylated recombinant factor VIII (BAX 855)

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Background: The frequency of bleeding events occurring in patients with severe hemophilia A (FVIII level <1%) can be controlled by the prophylactic use of FVIII. The safety and clinical efficacy of Recombinant Factor VIII (rFVIII)[#] and rFVIII covalently bound to PEG (BAX 855) were recently demonstrated in independent studies.

Aims: To compare individual annual bleeding rates (ABR) in severe hemophilia A patients treated prophylactically with rFVIII or BAX 855 in two clinical studies.

Methods: While traditional significance testing usually deals with differences between population averages, there is an increasing focus on comparing the probability of success of one treatment over another on a patient level. The relative treatment effect approach provides the probability that a randomly selected subject treated with BAX 855 has a higher ABR than a randomly selected subject treated with rFVIII. This probability is denoted as relative treatment effect and equals 0.5 if both groups are stochastically equal. Thus, an estimate for the relative treatment effect and its 90% confidence interval (90% CI) of pro-

phylaxis with BAX 855 and rFVIII on ABR was calculated according to Brunner & Munzel (Biometrical Journal, 2000). The margins of equivalence were set to 40% to 60% as suggested by Wellek (Chapman & Hall, 2010).

Results: The ABR analysis included 55 subjects treated with rFVIII (20–40 IU kg⁻¹ every 2 days; or 20–80 IU kg⁻¹ every 3 days) and 120 subjects treated with BAX 855 (40–50 IU kg⁻¹ every 3–4 days). The point estimate for the relative treatment effect of BAX 855 to rFVIII on ABR was 52.1%. The 90% CI for the relative treatment effect was 44.6% to 59.5% and thus completely included within the margins of equivalence, demonstrating equivalence in the 'strict' sense of Wellek.

Conclusion: The relative treatment effect of prophylactic treatment with BAX 855 and rFVIII on ABR in patients with severe hemophilia A demonstrates their equivalence in bleeding prevention. #ADVATE.

Disclosure of Interest: W. Engl Employee of: Baxter, B. Abbuehl Employee of: Baxter, M. Wolfsegger Employee of: Baxter, B. Pavlova Employee of: Baxter, B. Ewenstein Employee of: Baxter.

PO255-WED

Low annualized bleeding rates (ABRS) with recombinant factor VIII FC fusion protein (RFVIII FC) across demographic subgroups of previously treated pediatric subjects with severe hemophilia A

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Background: The phase 3 Kids A-LONG study evaluated the safety and efficacy of rFVIII FC for prophylaxis and treatment of bleeding in previously treated children with severe hemophilia A.

Aims: To describe ABRs with rFVIII FC in demographic subgroups of Kids A-LONG participants.

Methods: The phase 3 Kids A-LONG study enrolled males < 12 years with severe hemophilia A (< 1 IU dL⁻¹ endogenous FVIII activity), ≥ 50 prior FVIII exposure days, and no history of inhibitors. Subjects received rFVIII FC prophylaxis 2x/wk (25 IU kg⁻¹ day 1, 50 IU kg⁻¹ day 4) for ~26 weeks, with adjustments to dose (≤80 IU kg⁻¹) and interval (≥ every 2 days) as needed. Prespecified sensitivity and subgroup analyses of ABR were conducted.

Results: Of the 71 subjects enrolled, 63 were on pre-study FVIII prophylaxis. Median (interquartile range [IQR]) on-study ABR was 1.96 (0.00, 3.96) overall, and 0.00 (0.00, 3.96) and 2.01 (0.00, 4.04) for subjects aged < 6 and 6 to < 12 years at screening, respectively. For subjects on study ≥24 weeks, median (IQR) ABR for the last 3 months on-study was 0.00 (0.00, 4.01). For subjects on pre-study FVIII prophylaxis, the median estimated number of bleeding episodes in the prior 12 months was 2.00, and the median on-study ABR was 1.96; for subjects on a pre-study FVIII episodic regimen, these values were 12.00 and 2.05, respectively. Assessed by bleeding history in the prior 12 months (pre-study bleeding episodes: 0 [n = 13 subjects], 1–5 [n = 37], 6–10 [n = 8], and >10 [n = 11]), median on-study ABR was 0.00, 1.96, 3.91, and 0.00, respectively. Thirteen (18.3%) subjects had ≥ 1 target joint at screening; median (IQR) on-study ABR was 0.00 (0.00, 5.83) for subjects with, and 1.97 (0.00, 3.87) for subjects without, target joints at screening.

Conclusion: Low rates of bleeding were observed in pediatric subjects treated with rFVIII FC regardless of pre-study bleeding phenotype or treatment, or the presence of target joints at screening.

Disclosure of Interest: G. Young Consultant for: Novo Nordisk, Biogen Idec, Baxter, Bayer, and Kedrion, Speaker Bureau of: Novo Nordisk and Biogen Idec, R. Kulkarni Grant/Research Support from: Biogen Idec, Novo Nordisk, and Octapharma, Consultant for: Biogen Idec, Novo Nordisk, Baxter, Pfizer, and Bayer, M. Ragni Grant/Research Support from: Baxter Bioscience, Baxter Healthcare, Bayer, Biogen Idec, CSL Behring, Novo Nordisk, and Pfizer, Consultant for: Biogen Idec, S. Brown Consultant for: Novo Nordisk, Biogen Idec, and Baxter, I. Woznica-Karczmarz Grant/Research Support from: Biogen Idec and Baxter, Speaker Bureau of: Novo Nordisk and Baxter, J. Puetz Grant/Research Support from: Biogen Idec, Speaker Bureau of: CSL Behring, R. Tamminga Grant/Research Support from: Bayer, G. Gambino Shareholder of: Biogen Idec, Employee of: Biogen Idec, G. Pierce Shareholder of: Biogen Idec, Employee of: Former employee of Biogen Idec, G. Allen Shareholder of: Biogen Idec, Employee of: Biogen Idec.

PO256-WED

Children with hemophilia in the US – the hemophilia associated caregiver burden scale (HEMOCAB™)

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Background: Caring for a child with a chronic disease can cause caregiver burden. No hemophilia-specific instrument to assess caregiver burden exists.

Aims: To conduct a pilot test using the 'Hemophilia associated Caregiver Burden Scale' (HEMOCAB™) with US caregivers.

Methods: Caregivers of children with hemophilia (CWH) with and without inhibitors < 22 years old were recruited at RUSH Hemophilia and Thrombophilia Center. Descriptive statistics shown as frequency distribution or as mean ± standard deviation, medians and ranges were tabulated for demographic data, clinical characteristics of CWH, and caregiver's burden assessed via HEMOCAB™. HEMOCAB™ consists of 13 domains and is scored on a scale of 0–100; higher scores correspond with greater burden. Student's t-test was used to assess differences between groups. Informed consent and IRB approval was obtained.

Results: A total of 40 caregivers were recruited; 75% were mothers (n = 30). Mean age of caregivers was 39.3 ± 8.9; mean age of CWH was 10.9 ± 5.5. The majority of caregivers were married (67.5%), had a child with hemophilia A (95%), and did not have a child with inhibitors (85%). Among CWH, 82.5% were on prophylaxis (n = 33), 50% self-administered factor (n = 20), and they experienced a mean 4.8 ± 8.9 bleeds in the prior year. Mean total score of HEMOCAB™ was 27.6 ± 20.0. The domains with greatest burden were 'perception of child' (37.9 ± 20.6), 'emotional stress' (35.7 ± 23.3) and 'financial burden' (33.9 ± 30.0). There was a significant difference in mean scores among caregivers of CWH with inhibitors (past or current) compared to caregivers of CWH without inhibitors, in all domains, except 'school'. No differences were observed based on number of bleeds experienced in the prior year.

Conclusion: Pilot testing suggests caregiver burden for hemophilia is greater among those whose child ever had inhibitors. Implementation of HEMOCAB™ in a larger population will increase robustness of data to discern differences between groups.

Disclosure of Interest: S. von Mackensen Consultant for: Novo Nordisk Inc., T. Wisniewski Employee of: Novo Nordisk Inc., J. Urgo Grant/Research Support from: CSL, Bayer, Baxter, Novo Nordisk, OctaPharma, Selexis, Consultant for: Bayer, Baxter, Novo Nordisk, Biogen, L. Boggio Grant/Research Support from: CSL, Bayer, Baxter, Novo Nordisk, OctaPharma, OPKO, Selexis, Consultant for: CSL, Bayer, Baxter, Novo Nordisk, OctaPharma.

PO257-WED

Developing a measure of hemophilia caregiver impactSchwartz C^{1,2} and Eldar-Lissai A³¹*Medicine and Orthopaedic Surgery, Tufts University School of Medicine, Boston;* ²*Delta Quest Foundation, Concord;* ³*Global Market Access, Biogen Idec, Cambridge, USA*

Background: Caring for someone with hemophilia is a demanding role. Usually diagnosed at birth or soon thereafter, hemophilia is a life-threatening condition that requires constant vigilance to prevent and/or identify bleeds, and continual proactive medical management. Although the impact of hemophilia on the patient and family is clearly important, there is currently no validated measure of hemophilia caregiver burden.

Aims: To develop an item bank of questions measuring hemophilia caregiver impact, and to establish the content validity of this item bank.

Methods: We implemented a review of the literature on caregiver burden to identify a useful conceptual model of the construct, and then reviewed existing caregiver-burden measures for other conditions (e.g., cancer, asthma). We drafted items drawing from these existing measures, with an emphasis on maintaining a consistent response-option format for ease of use. We then pretested the item bank for clarity and content coverage in interviews with current hemophilia caregivers.

Results: Our conceptual model identified the following caregiver impact dimensions as relevant: practical support, symptoms of hemophilia, lifestyle, social, physical, emotional, financial, and positive emotions. Three rounds of pretesting interviews (n = 22) included 20 female and 2 male caregivers, age 29–58. Our initial item bank of 126 questions was modified, expanded, and contracted to yield a final item bank of 105 questions, with subsets of items intended for subgroups of caregivers for the newly diagnosed, and for infants/young children, and adolescents/young adults. Feedback from interviewees supports the content validity and clarity of the item bank, which is estimated to take 15 min to complete.

Conclusion: The Hemophilia Caregiver Impact Measure covers content relevant to hemophilia caregiver burden, and is transparent and feasible. Further testing is required to evaluate the measure's reliability, validity, and responsiveness.

Disclosure of Interest: C. Schwartz Consultant for: Paid consultant to plan and implement the study., A. Eldar-Lissai Shareholder of: Biogen Idec, Employee of: Biogen Idec.

PO258-WED

Venepuncture vs. laboratory sample timing discrepancies: implications for pharmacokinetic prediction of prophylaxis dosing in severe hemophilia A: UK-PK pilot studyBatty P¹, Spencer Chapman MH¹, Platten S² and Hart DP¹¹*The Royal London Hospital Haemophilia Centre, Barts and The London School of Medicine and Dentistry;* ²*The Royal London Hospital Haemophilia Centre, Barts Health NHS Trust, London, UK*

Background: Population based pharmacokinetics (PK) allows estimation of individuals' PK profiles from a limited number of blood samples. This can guide dosing of prophylaxis in severe hemophilia A (HA). These strategies rely on data accuracy: FVIII:C; dose; infusion/sample timing. Variability within the electronic patient record (EPR) (actual venepuncture vs reported assay time) is not known. We hypothesise that timing discrepancy results in errors in estimation of PK and dose.

Aims: To evaluate the discrepancy between actual and reported time for FVIII/IX assays and model the effect on dose predictions for simulated severe HA patients.

Methods: Venepuncture times for FVIII/IX samples were recorded prospectively (10/14-11/14) and paired with EPR times. The effects of timing discrepancies at 2 sample points (6 and 12 h) were modelled in simulated patients with short (5th centile: 7.5 h), median (10.4 h) or long (95th centile: 16.5 h) half-lives (t_{1/2}) using Bayesian PK guided dosing software (myPKFit, Baxter Healthcare, US).

Results: 96 samples (44 patients) were sent for testing. The median timing discrepancy was 47 mins (-56 to 205 mins), with a discrepancy of >1 h in 44% (n = 42). The predicted dose for a 70 kg patient with median t_{1/2} was 1267 IU (18.1 IU kg⁻¹) every 48 h to maintain FVIII:C > 1%. Timing discrepancies (-1 and +3 h) at both sample points resulted in t_{1/2} predictions from 10.0 to 11.9 h with resultant dose predictions from 1428 (21.2 IU kg⁻¹) to 780 IU (11.1 IU kg⁻¹) respectively. Discrepancies of >1 h result in dose options with alternative vial size choices. The effects of these discrepancies are more marked in patients at the 5th and 95th centiles with predictions offering greater changes to dose or frequency.

Conclusion: With the introduction of population based PK guided dosing into clinical practice, centres must understand and adapt local pathways to ensure data accuracy. Inaccuracies may affect predictions of prophylaxis and result in over or under dosing of factor replacement.

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PO259-WED

A novel missense mutation, P.PHE360CYS, in F9 gene results in hemophilia B in female patient

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Background: Female patient with hemophilia was rare, and easy to be misdiagnosed.

Aims: To analyze the phenotype and genotype, and investigate the molecular mechanism of the female patient with hemophilia B.

Methods: A female patient complained of intracranial hemorrhage, with a history of menorrhagia, postpartum bleeding. Coagulation parameters were detected in the proband and her parents who were not consanguineous marriage. All exons of F9 gene were sequenced. A expression plasmid carrying c.36029T>G mutation was constructed and transiently transfected into HEK293 cells. The X chromosome inactivation pattern(XIP) was determined by PCR analysis.

Results: It was showed that a prolonged APTT(62.2s), decreased FIX: C (2%), normal PT and FVIII:C, with negative FIX inhibitor in proband. All coagulation parameters of her parents were normal. A novel missense point mutation (in exon 8 of F9 gene, c.36029T>G, p.Phe360Cys) was found in heterozygous state in proband, but not found in her parents. The antigen of FIX in proband was the same as the normal person by western blotting. The antigen level of F9MT (c.36029T>G, p.Phe360Cys) was similar to the level of wild type in media and cell lysates in vitro. The study of X-chromosomes inactivation analysis is still going on.

Conclusion: We identified a female patient with hemophilia B, due to a novel missense mutation (c.36029T>G, p.Phe360Cys) in F9 gene. Located in its catalytic domain, this mutation impaired the FIX activity, but didn't affect the synthesis and secretion of FIX, which had been confirmed by transfection tests in HEK293 cells. Because this mutation in F9 gene didn't be detected in her parents, we postulated that this mutation may originate from her father's spermatogonium, and adding to X-chromosomes inactivation derived from her mother, all of which finally resulted in phenotype of hemophilia B in proband.

Disclosure of Interest: None Declared.

PO260-WED

Inhibitors in previously untreated patients (PUPS) with severe hemophilia A: a follow-up cohort study

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Background: Inhibitor (INH) development is the main complication of treatment in children with severe hemophilia A (HA) rendering bleeding control difficult and prophylaxis unfeasible. Little is known about the natural history of INH in children and the associated bleeding phenotype. Moreover immune tolerance induction (ITI) still needs to be optimized in this patient group.

Aims: This study is aimed at describing the natural history of INH and the bleeding phenotype in a cohort of previously untreated children with severe HA as well as the use of ITI and its outcome.

Methods: The study is a satellite of the PedNet Registry including all children who developed INH and for whom a 3-year follow-up after inhibitor development was available. Data collection pertains 3 periods: pre-, during and after ITI. Data on bleeding frequency, treatment of bleeding episodes, type and site of bleeding and details on ITI (regimen, doses, product) have been collected.

Results: Data are available from 259 children who developed clinically relevant INH (186 high-responders, 72%). The median age at INH development was 1.3 years (IQR 0.9-1.7). The median INH titer at diagnosis was 3 BU mL⁻¹ (IQR 1-13) and the median historical peak 19 BU mL⁻¹ (IQR 4-110). Up to date complete treatment data for the 3-yr follow-up after INH development is available for 224 children (86%) of whom 197 started ITI (88%). The median time elapsed between INH development and ITI start was 1.1 month (IQR 0.03-8.1). At the end of the 3-yr follow-up 103 patients completed ITI and the median duration of ITI was 13.3 months (IQR 7.8-22.2). Data collection and analysis on ITI regimens and outcome and on bleeding phenotype prior, during and after ITI is ongoing.

Conclusion: Interim analysis demonstrated that 88% started ITI after a median time period of 1.1 months. The data of this large unselected cohort will shed new light on bleeding phenotypes of low and high titer INH and the success rate of ITI.

Disclosure of Interest: None Declared.

PO261-WED

Satisfaction patient survey: a key tool to improve patient delivery of care at a Portuguese Comprehensive Hemophilia Centre

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Background: Patient satisfaction is recognized as a key component of health-care delivery and an indicator of health-care quality, mainly in comprehensive hemophilia care centres, where patients are long-term users.

Aims: To evaluate patient satisfaction through a survey adapted with permission from a previously validated questionnaire (Makris, Sheffield), to guide decisions regarding health-care services and evaluate satisfaction with competence, quality of care and access.

Methods: A patient satisfaction questionnaire was mailed to 69 patients with hemophilia A or B, severe or moderate, all ages. The questionnaire had fixed-choice questions regarding demographical characteristics, patient satisfaction with services provided by staff cen-

tre and other specialties related to comorbidities, with options ranging from always to never, strongly agree to strongly disagree or does not apply and at the end, there were open questions asking patients opinion and improvement suggestions

Results: The response rate for the entire cohort was 39% (27/69). The two main groups of returned surveys were from patients less than 18 and 40-59 years. A SWOT analysis was performed: our strength is a very high patient satisfaction with the centre staff and services provided, such as staff knowledge about disease/treatment, waiting time less than 30 min and presence of doctor/nurse 24 h day⁻¹; our weakness was related to services provided outside our centre: patient parking, other medical speciality consultations and waiting time to get home treatment at the pharmacy; we identified the opportunity to improve services outside and the threat of hypothetical economic constraints, downgrading delivery of care.

Conclusion: Patient satisfaction may be an important determinant of adherence to difficult treatment regimens, such as prophylaxis. The information received from this survey has been helpful in guiding decisions regarding our comprehensive centre and improving provided outside services.

Disclosure of Interest: None Declared.

PO262-WED

Numerical models of thrombin generation applicable to hemophilia

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Background: Determination of an individual clotting potential by Thrombin Generation (TG) assay could permit individualized treatment for hemophilic patients. Many kinetic numerical models of TG have been developed, but before their use for treatment individualization, they must first be validated for hemophilia.

Aims: To assess several numerical TG models in hemophilic (H) patients.

Methods: Experimental data were obtained from 40 HA and 32 HB patients by measuring TG in Platelet-Rich Plasma (PRP) and Platelet-Poor Plasma (PPP) using the CAT system (Hemker, Pathophysiol Haemost Thromb, 2002) with a 1 pM tissue factor concentration. Clotting factors and inhibitors were measured by the usual chronometric or colorimetric methods. TG models were selected from available literature according to their correspondence to real TG assays and implemented in Matlab. Experimental factors and inhibitor levels constituted the inputs. The calculated (output) and corresponding experimental TG data were compared with respect to Lag Time, Endogenous Thrombin Potential (ETP), Peak, Start Tail, and Velocity Index. Model predictions were assessed using the Stone-Geisser criterion Q2 and then optimized by applying a genetic algorithm to the model kinetics. Results: In all of the models implemented in their original state, the Q2-criterion indicates a lack of validity on almost all the parameters assessed. The best agreements between model and experimental data were obtained with the models developed by Panteleev (Biophys. J., 2010) and Chatterjee (PLoS Comput Biol, 2010). Model optimization resulted in a pertinent improvement in prediction, especially in the Panteleev model, leading to a decrease of the mean squared error on ETP by a ratio of 3.94 for HA in PRP and a ratio of 3.28 for HB in PRP.

Conclusion: Predictions based on current numerical models diverged substantially from experimental data. Optimization of their kinetics by integrating experimental TG data seemed to improve their accuracy.

Disclosure of Interest: None Declared.

PO263-WED

The effect of Nordic Walking on joint status, quality of life, physical ability, exercise capacity and pain in older men with hemophiliaSalim M¹, Späls-Abrahamsson Y¹, Berntorp E¹, Brodin E² and Zetterberg E¹¹*Translational Medicine, Skane University hospital, Lund University, Malmö;* ²*Institute of Neuroscience and Physiology, Section for Clinical Neuroscience and Rehabilitation, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden***Background:** Regular exercise has been shown to improve muscle strength and bleeding profile in children and young adults with hemophilia, but little is known of the effects of exercise in adult patients. Nordic Walking is a highly effective form of training where the use of walking poles increases energy consumption and improves balance.**Aims:** The aim of this study was to examine the effect of 3 months of Nordic Walking in patients with hemophilia > 40 years of age, regarding joint function, physical ability, exercise capacity, pain and quality of life.**Methods:** After informed consent 10 patients were required to perform 30 min of Nordic Walking, at least 3 times a week, during a period of 3 months and were motivated to continue for additionally 3 months. The pain level before and after the workout, the joint health (HJHS 2.1) and the general exercise capacity was evaluated before and after the 3 months training period. Evaluation of physical ability (HEP-Test-Q), quality of life (SF-6) and kinesophobia (The Tampa Scale of Kinesophobia), were evaluated before, after the three months training period as well as after 3 months of additional follow up. Differences in pre- and post-interventional scores were analyzed using Wilcoxon Signed Ranks Test.**Results:** The participants exercised on average 43 min 2.4 times a week. The intervention did not influence quality of life, pain or joint health status. Statistically significant improvements were observed in physical ability, $P = 0.01$, and in general and body perception, $P = 0.02$, assessed by HEP-Test-Q.**Conclusion:** This study, being the first study ever examining the effects of Nordic walking in hemophilia, shows that Nordic Walking is a safe exercise form, resulting in improvements in physical ability and in general and body perception already after 3 months. We hypothesize that changes in quality of life, joint health status and pain-levels require longer training periods and/or a larger study cohort.**Disclosure of Interest:** M. Salim: None Declared, Y. Späls-Abrahamsson: None Declared, E. Berntorp: None Declared, E. Brodin: None Declared, E. Zetterberg Grant/Research Support from: Baxter.

PO264-WED

Perioperative management and outcome of urgent appendectomy in a patient with severe hemophiliaSöker M¹, Zeytun H², Söker S³ and Şahin C¹¹*Pediatric Hematology and Oncology;* ²*Pediatric Surgery;* ³*Histology and Embryology, Dicle University Medical Faculty, Diyarbakir, Turkey***Background:** Perioperative administration of Factor VIII reduces post-operative hemorrhagic complications in patients with hemophilia A, who were undergoing elective surgical procedures. However, there are few guidelines for the perioperative management of hemophilia A patients undergoing emergent surgical procedures. We report the presentation of acute appendicitis in an 11-year-old boy with severe hemophilia A. We managed the case according to recommendations regarding factor VIII replacement in perioperative period to ensure adequate hemostasis.**Aims:** An 11-year-old boy with severe hemophilia A admitted to pediatric emergency unit with acute onset of abdominal pain. The pain began in the right lower quadrant. The pain persisted for an additional 12 h and complain of nausea and emesis. Physical examination findings revealed tenderness to palpation in the right lower quadrant with rebound tenderness. Laboratory studies revealed, white blood cell count 21.19×10^3 /mL, hemoglobin 11.8 g dL^{-1} , factor VIII level 0.9%, and factor VIII inhibitor within normal limits. Radiologic imaging studies abdomen and pelvic ultrasonography was performed to determine a dilated appendix with adjacent mesenteric edema. The patient was given recombinant factor VIII (2000 U; 50 IU per kilogram of body weight) immediately before surgery. The patient underwent open appendectomy without complication. His appendix was acutely inflamed. Intraoperative bleeding was minimal. Then recombinant factor VIII continued every 12 h for 7 days. Patient did not experience any bleeding recurrence during this period. The patient was discharged with prophylactic recombinant factor VIII replacement therapy. No recurrent haemorrhage or complications were occurred.**Methods:** –**Results:** –**Conclusion:** Appropriate treatment is essential for full recovery following general surgical procedures of patients with severe hemophilia. The perioperative care of a patient with hemophilia requires a coordinated effort that includes the surgeon and hematologist.**Disclosure of Interest:** None Declared.

PO265-WED

Prevalence, severity and correlation factors of hemophilic arthropathy among Taiwanese hemophiliacsChang C-Y^{1,2,3}, Li T-Y^{4,5}, Cheng S-N^{5,6}, Pan R-Y^{5,7}, Wang H-J^{3,8}, Lin S-Y^{3,9} and Chen Y-C^{5,10}¹*Department of Pediatric Hematology/Oncology, Taipei Medical University Hospital;* ²*School of Medicine, Graduate Institute of Clinical Medicine, Taipei Medical University;* ³*Hemophilia Center, Taipei Medical University Hospital;* ⁴*Department of Rehabilitation;* ⁵*Hemophilia Center, Tri-Service General Hospital;* ⁶*Department of Pediatrics, Tungs' Taichung MetroHabor Hospital;* ⁷*Department of Orthopedics, Tri-Service General Hospital;* ⁸*Department of Medical Imaging;* ⁹*Department of Intern Medicine, Taipei Medical University Hospital;* ¹⁰*Department of Hematology/Oncology, Tri-Service General Hospital, Taipei, Taiwan***Background:** Hemophilic arthropathy (HA) is a major complication of patients with hemophilia (PWH), although its prevalence can be reduced with prophylaxis. The prophylaxis therapy for pediatric PWH was implemented in Taiwan after 1995.**Aims:** We aim to analyze retrospectively the prevalence, severity, and correlation factors of HA among Taiwanese PWH.**Methods:** From 2006 to 2014, 146 severe/moderate-type PWH (134 hemophilia A, 12 hemophilia B) were enrolled, with median age of 33 (4–73). The data including disease severity, prophylaxis therapy, BMI, and HCV infection were collected. HA was assessed by X-ray films of the 6 index joints (elbows, knees, ankles) and scaled by Pettersson score.**Results:** The age group-specific prevalence of HA was 42.8% in 4–10 year-old PWH, 64.3% in 11–19 year-old PWH, 97.1% in 20–29 year-old PWH, 93.1% in 30–39 year-old PWH, and 100% in PWH with ≥ 40 y/o. The average joint number and Pettersson score of HA was 1.3 and 1.9 in 4–10 year-old PWH, 1.4 and 4.1 in 11–19 year-old PWH, 2.9 and 15.6 in 20–29 year-old PWH, 4.4 and 33.1 in 30–39 year-old PWH, 5.1 and 46.1 in 40–49 year-old PWH, 5 and 49.6 in

PWH with ≥ 50 years old. For PWH with age ≤ 10 years old, a half had bilateral HA of ankle, and none of them had HA of knee. Severe HA were less found in < 20 year-old PWH. In PWH with < 40 y/o, prevalence of HA by location was ankles $>$ elbows $>$ knees. In PWH with ≥ 40 y/o, severe HA of elbow and knee accounted larger ratio than that of ankle. By univariate analysis, age (≥ 18 y/o), severity, HCV infection, and prophylaxis therapy were significantly associated with total Pettersson score. ($P < 0.05$) Both age and HCV infection had positive correlation with severity of HA. ($r > 0.7$, $P < 0.01$) Only age and severity were the independent factors by multivariate analysis.

Conclusion: There was high prevalence of HA in Taiwanese PWH, especially in adult PWH. In pediatric PWH, HA of ankle was more common than other joints. With the implementation of prophylaxis therapy, the prevalence and severity of HA in PWH have been much improved.

Disclosure of Interest: None Declared.

PO266-WED

Safety, pharmacokinetics and pharmacodynamics of PF-05280602 (recombinant FVIIA variant): preliminary results from a single ascending dose phase I study in hemophilia A and B subjects

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Background: PF-05280602 is a variant of activated recombinant human factor VII (FVIIa) in development for treatment and prevention of bleeding in hemophilia patients with inhibitors to factor VIII or factor IX. In hemophilic dogs it is characterized by greater potency and longer duration of pharmacodynamic effect relative to wild type FVIIa/eptacog alfa (1).

Aims: Investigate safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of a single dose bolus intravenous infusion of PF-05280602 in non-bleeding hemophilia subjects.

Methods: Male subjects aged 18–65 years with hemophilia A or B (factor activity $\leq 1\%$), with or without inhibitors to factor VIII or IX were enrolled. 24 subjects were assigned to four ascending single dose cohorts (4.5, 9, 18, or 30 $\mu\text{g kg}^{-1}$ of PF-05280602). Study assessments included safety monitoring, PK, PD, and immunogenicity testing. The study was approved by local ethics committees. Subjects provided written informed consent.

Results: A dose-dependent decrease in clearance and volume of distribution was observed. Half-life was approximately 3.5 h. Dose-dependent shortenings of activated partial thromboplastin time and prothrombin time were observed at all 4 dose levels. Treatment-induced increase of prothrombin fragment 1 + 2 was observed at higher doses. Shortening of lag time and increase of peak thrombin from thrombin generation assay were also observed at all doses. PF-05280602 was tolerated at all dose levels tested and was not associated with dose limiting toxicity. There were no treatment emergent serious adverse events. No subject developed anti-drug or neutralizing anti-

bodies. Data are preliminary since the study is ongoing for last subject immune follow up.

Conclusion: PF-05280602 was tolerated at doses up to 30 $\mu\text{g kg}^{-1}$. The safety profile and pharmacologic effects observed support further development of PF-05280602 for treatment of individuals with hemophilia and inhibitors to factor VIII or IX.

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PO267-WED

Severe hemophilia A patients who don't bleed while on prophylaxis: investigation into possible factors influencing the outcome

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Background: In ongoing Guardian™ clinical trials of turoctocog alfa (NovoEight®), a recombinant factor VIII product, previously treated patients (Pts) with severe haemophilia A (HA) were on prophylaxis. Interestingly, a large variation in annualised bleeding rates (ABRs) was observed with significant subpopulation having ABR of 0 to 1.

Aims: The aim of the present analysis was to investigate the potential influence of various patient factors including age, treatment regimen and compliance on the variation in ABR.

Methods: Patients (N = 181) with severe HA (FVIII activity $\leq 1\%$) without inhibitors, received prophylactic therapy with scheduled preventive turoctocog alfa injections (20–50 IU kg^{-1} once every second day or 20–60 IU kg^{-1} three times weekly) with additional treatment for bleeds. All patients with data as of 31 Dec 2013 with at least 1 year treatment duration were included in this analysis.

Results: For spontaneous bleeds, 55 Pts had ABR=0, 46 Pts had $0 < \text{ABR} \leq 1$ and 80 Pts had $\text{ABR} > 1$. For all bleeds, there were 21 Pts who did not bleed at all (ABR=0), 34 Pts had $0 < \text{ABR} \leq 1$ and 126 Pts had $\text{ABR} > 1$. For spontaneous bleeds, in the subgroups $\text{ABR} = 0$ vs $0 < \text{ABR} \leq 1$ or $\text{ABR} > 1$, the mean duration in the trial, 2.8 vs 2.9 or 2.9 years; mean weekly dose, 96 vs 93 or 93 IU kg^{-1} Body weight; the mean proportion of life on prophylaxis, 38.0 vs 33.6 or 29.5% and compliance expressed as mean sum of days since last dose for late doses per patient (28.2 vs 30.1 or 33.9) were comparable. There was a trend for patients with lower spontaneous ABR to be younger (mean age, 16.00 vs 20.5 or 23.9 years) at entry into the Guardian™ trials. No correlations were observed for ABR covering all bleeds.

Conclusion: Based on the observed data, a considerable variation in the ABR of patients with severe HA on prophylaxis could not be attributed to age, weekly dose or compliance. Further investigations may identify factors that lead to the absence of bleeding episodes in some severe HA patients during prophylaxis.

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PO268-WED

Quality of life among haemophilia in Thailand

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Background: Thai national health policy has developed the accessibility to factor VIII concentrate for early home therapy among Thai patients with haemophilia (hph) for years which should improve their quality of life (QoL). However, data on hph patients' QoL are scarce.

Aims: To study the QoL among Thai hph patients and to study what the hph patients affect their families and care givers on social, financial, and relationship aspects.

Methods: Questionnaire and consent form were sent to 1135 hph patients in Thailand (696 adults & 439 children) by mail. We used the haemophilia-specific health-related quality of life questionnaire (HAEMO-QoL-A; 41 items) for adults aged above 16 years, HAEMO-QoL-kids (35 items) for children aged 8–16 years and a separated questionnaire for parents or caregivers. Scoring system for Haemo-QoL-A showed the score ranging from 0 to 100 in which the higher score represented the better QoL. For Haemo-QoL-kids, the higher score (the score was 0–100) meant more deformity and worse QoL. Results: A total of 272 patients (228 adults and 44 children) sent back the filled questionnaires. The median age among adult and children responders was 27 (range 16–67) and 12 (range 8–16), respectively. The number of mild, moderate and severe hph among adults were 58, 68, and 94 (8 missing data), respectively, and among children were 6, 23, and 11 (4 missing data). The total mean score of HAEMO-QoL-A was 51.2 ± 12.3 which was not different among severity of hph. For the domain of worry, severe hph adults had a significantly lower score than mild hph (43.97 vs 55.97; $P = 0.037$). The total mean score of HAEMO-QoL-kids was 45.7 ± 13.6 which did not differ among severity of hph. For the domain of sport, severe hph children had a significantly higher score than moderate hph (81.0 vs 61.0; $P = 0.028$). Majority of hph family (55%) had a financial problem.

Conclusion: The quality of life of haemophilia patients in Thailand was impaired in all aspects and the financial problem is common for the parents and care givers.

Disclosure of Interest: None Declared.

PO269-WED

Impact of transition on clinical outcomes in hemophilia: 10 years of experience at a major canadian treatment center

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Background: Hemophilia is a chronic disease best managed in interdisciplinary comprehensive care settings. In British Columbia (BC), persons with hemophilia (PWH) transition from a pediatric to adult centre upon adulthood. Literature has focused on self-efficacy and transition preparation; little data exists on clinical outcomes during and after transition.

Aims: To describe population transitioned, transition gap between clinics, assess bleeding outcomes before and after transition for those with severe hemophilia in BC.

Methods: Retrospective review of patients who transitioned in 2004–2014 using standardized data collection procedures. Subjects were male, followed ≥ 5 years in pediatric and ≥ 3 years in adult centre with ≥ 1 post-initial intake visit. Transition gap period was defined as the interval between last pediatric and first adult clinic visit.

Results: 63 subjects were referred (57% severe 13% mod 30% mild) with 26 subjects included (73% severe 19% mod 8% mild). Excluded patients did not have adequate time in clinic (27/37) for outcome assessment or were lost to follow-up (10/37, 50% of these mild). Median gap between pediatric and adult clinics was 8.5 months. 72% with severe hemophilia (excluding 1 inhibitor) showed an increased mean annual bleed rate by 3.9-fold during the gap; this did not return to baseline in the 3 years post transition and was observed for muscle and joint bleeds. These patients were on long-term prophylaxis for a median of 12 years; 1 patient discontinued prophylaxis post-transition. Factor consumption ($u\text{ kg}^{-1}\text{ year}^{-1}$) was stable during the gap.

Conclusion: Patients with severe hemophilia in BC are seen within 1 year of exiting the pediatric program; over half were observed to bleed more during and after the transition gap. Follow-up is needed with the excluded group, particularly those lost to follow-up. In centres with distinct pediatric and adult clinics, earlier visits to the adult clinic (i.e. 1–3 months) may be warranted to follow clinical outcomes more rigorously during transition.

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PO270-WED

Pro- and anticoagulant factors balance thrombin generation induced by APCC

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Background: APCC is comprised of the zymogen forms of the procoagulant factors: II (prothrombin), VII, IX, X, with trace amounts of their activated form, anticoagulants including protein C and tissue factor pathway inhibitor (TFPI). These are present in a physiologically balanced ratio. Small amounts of cofactor proteins are also present: factors V, VIII, and protein S.

Aims: It was found previously that a complex of F II and F Xa has a key, but not exclusive role in the mode of action of FEIBA. The supporting roles of the other protein components of APCC were systematically investigated by measuring their *in vitro* modulating effect on thrombin generation in high titer F VIII inhibitor plasma.

Methods: Purified hemostatic factors were mixed together in a ratio corresponding to those in APCC and spiked in the factor VIII inhibitor plasma in a concentration matching that present in 1 APCC U/mL. Thrombin generation was triggered by low tissue factor (TF) and phospholipid (PL) concentration, which simulates the *in vivo* situation on the platelet surface at small vessel injuries.

Results: Factor VIII-deficient plasma with inhibitor showed nearly no thrombin generation when triggered with a low concentration of TF-PL complex. The addition of APCC to the inhibitor plasma sample improved the thrombin generation in a concentration-dependent manner; normal range was achieved at plasma concentrations above 0.5 APCC U mL⁻¹. Investigation of the isolated enzyme-substrate complex of F II and FXa revealed that the two proteins act in synergy. Thrombin generation mainly depended on FXa concentration, addition of the zymogen factors increased the peak thrombin and decreased the lag time. However, when protein C and Protein S were added, a slower thrombin generation with less peak thrombin was detected. TFPI in concentrations found in APCC did not alter thrombin generation to a relevant extent.

Conclusion: In summary it was shown that all components of APCC are required for the optimal burst of thrombin generation.

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PO271-WED

Muscle strength in load-bearing joints in haemophilic patients: comparative values with the general population

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Background: The knee haemarthrosis is the musculoskeletal injury that most often affects the haemophilic patient and is the basis of haemophilic arthropathy. Muscle strength is a basic quality in the physical condition and it is part of the exploratory items that allow diagnosis and supervision of arthropathy.

Aims: Compare isometric strength of the haemophilic children knee extensors with the general population.

Methods: Isometric quadriceps strength in children from 9 to 10 years using a portable dynamometer (Nicholas MMT, Lafayette Instruments) was evaluated. The dynamometer was placed in the distal leg as close as possible to the ankle. The peak force (kg) was registered 3 times on each leg for 6s and the measured was dividing by the subject weight (kg) for standardization.

Results: A total of 19 haemophiliacs (16 severe in prophylaxis and 3 mild on demand) with a mean rating of 0.45 points in knees and 0.42 points in ankles (HJHS score), were evaluated. Their average height is 143.9 cm, weight 35.0 kg and BMI 16.84 kg m⁻². Regarding the physical activity, 13 children do sport regularly (61.5% swimming, 31% soccer, 7.5% other). As a control group, 19 children without hemophilia were evaluated. Their average height is 138.2 cm, weight 34.7 kg, BMI 18 kg m⁻² and 14 do sports (57% soccer and 43% other).

Regarding the force, the haemophilic group achieved a mean value of 0.66 (right leg) and 0.67 (left leg). The control group achieved a value of 0.86 (right leg) and 0.83 (left leg). Data normality was tested (Lilliefors test) and then a two sampled t-Test was performed. Results indicated statistically significant differences in the mean strength of both groups ($P = 0.008$ and $P = 0.020$).

Conclusion: To prevent musculoskeletal problems and improve quality of life, muscle strength is important because it provides stability and joint protection. Our results show significant differences from the control group. These data justify the need to expand studies and adopt measures associated with haematologic physiotherapy treatment.

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PO272-WED

Personalized prophylaxis with Human-CL RHFVIII in hemophilia A patients

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Background: Prophylactic therapy is aimed to maintain sufficient FVIII plasma levels to prevent bleeding. Trough FVIII level and time of FVIII level >1% are mainly determined by the half-life of the FVIII concentrate and the injection interval.

Aims: A clinical study was conducted to investigate the efficacy of individually PK-tailored prophylaxis with Human-cl rhFVIII.

Methods: This prospective, open-label, multicentre phase 3b study included previously treated adult patients with severe hemophilia A. Each patient started with a PK evaluation followed by 1-3 months of routine prophylaxis (Phase I) until individual PK data were analyzed. It was calculated, which dose and injection interval would theoretically result in a trough FVIII level of $\geq 1\%$. Then, patients continued prophylaxis for another 6 months based on the individually recommended treatment schemes (Phase II). The study was approved by the ethics committees and informed consents were obtained.

Results: Key results on the first 30 of the 66 patients who completed the study are: The prophylactic injection interval was extended from usually 3 times per week in Phase-I to twice per week or less in 53% of patients for phase-II.

- The median dosing interval in phase-II was 3.5 days.
- 77% of patients did not experience any bleeding episode during phase-II.
- The mean annualized bleeding rate (ABR) in phase-II was 1.39, median 0 (2.0, 0 in phase I).
- The dose per week needed for prophylactic treatment in phase-II was about 10% lower than in phase-I.

- There were no inhibitors and related adverse events.

Final results on all patients will be submitted as soon as available, and presented at the meeting.

Conclusion: The data suggest that personalized prophylaxis with Human-cl rh FVIII results in a more convenient treatment for more than half of the patients with lower factor consumption and a lower ABR while remaining safe and efficacious.

Disclosure of Interest: None Declared.

PO273-WED

Thromboprophylaxis for major orthopedic surgery in hemophilia: outcomes of an expert opinion survey on clinical practice and applicability of ACCP guidelines by the ADVANCE Working Group

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Background: Indications and modalities of thromboprophylaxis in patients with hemophilia (PWH) undergoing major orthopaedic surgery remain controversial.

Aims: We aimed to identify risk factors for venous thromboembolism (VTE), optimal approaches of thromboprophylaxis and applicability of American College of Chest Physicians (ACCP) guidelines to PWH undergoing hip or knee arthroplasty (THR, TKR).

Methods: A survey was performed among 21 members of the ADVANCE Working Group, who had undertaken 82 THR, 189 TKR and 41 ankle arthrodesis over the last 24 months.

Results: In patients with severe and moderate hemophilia, major orthopedic surgery was not considered as a risk factor for VTE per se (76%), at variance with was perceived for mild hemophilia (48%). Thromboprophylaxis was used by 24% and 47% of the treaters for patients with severe/moderate and mild hemophilia, respectively. Local guidelines for thromboprophylaxis were used in 33% of centers. Pharmacological thromboprophylaxis was considered to increase the risk of bleeding in more than 50% of centers. Mechanical thromboprophylaxis was preferred over pharmacological approach (62%). Factors affecting the decision to provide pharmacological thromboprophylaxis were: personal or family history of VTE, known thrombophilia, active cancer, mild hemophilia, history of major bleeding, hemophilia B (with other risk factors). Most ACCP guidelines on pharmacological prophylaxis were not considered to be applicable for PWH; however, exceptions were the preferential use of LMWH, the use of intermittent pneumatic compression devices or 'no prophylaxis' rather than pharmacological treatment.

Conclusion: Thromboprophylaxis is currently used by a limited number of centers. Specific risk factors were identified that could justify thromboprophylaxis in a subset of patients. Only a very limited number of the ACCP practical guidelines of pharmacological prophylaxis are applicable to PWH.

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Disclosure of Interest: None Declared.

PO274-WED

Age and treatment related influences on annual bleed rates (ABR) in patients with hemophilia A (PWA): observational study data of patients treated with recombinant FVIII formulated with sucrose (rFVIII-FS) in a global setting

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Background: Half-life of FVIII in PWA increases with age. How this affects bleed rates in real life remains unclear.

Aims: This study aims to investigate the association of ABR with age and treatment regimen in PWA treated with Bayer's rFVIII-FS in a real life situation.

Methods: After ethics approvals, consenting PWA were followed for at least 24 months (Latin America n = 92, International 155, Taiwan 69, Germany 176) from 2008 to 2013. Apart from descriptive statistics by age category, severity of hemophilia and treatment regimen, a multivariate analysis explored the association of age, severity, geography, treatment regimen, pretreatment, joint status, comorbidities, inhibitor status with the annualized total (ABR) and joint bleed rates (JBR).

Results: 492 PWA had infusion diaries available of whom 80% were severe PWA ($\leq 2\%$ FVIII:C). Mean age was 20.4 (range 0-75) years (y) with the following age distribution: [≤ 12 years] 43%; [12-18 years] 16%, [18-40 years] 24%, [≥ 40 years] 18%. 12.8% had an inhibitor history. 61% of children were on $\geq 1x/week$ pure prophylaxis (PP) (49% in adults). More adult patients (70%) had target joints than children (32%). Mean annual infused dose was 2775 ± 5266 IU kg⁻¹. Severe PWA on PP vs. on-demand (OD) had mean ABRs as follows: [All] 4.2 vs. 16.4; [≤ 12 years]: 4.7 vs. 18.9, [12-18 years]: 3.1 vs 12.7, [18-40 years]: 3.2 vs 15.0; [≥ 40 years]: 5.6 vs. 17.6. Respective JBR were: 2.2 vs. 11.8 (All), with lowest JBR (1.8 vs 11.6) for [18-40 years]. Multivariate analyses revealed rFVIII-FS prophylaxis treatment as the major predictor of the ABR ($P < 0.001$), surpassing all other influencing factors, e.g. severity of hemophilia ($P < 0.02$). Age showed a significant inverse association with JBR ($P = 0.04$), but only a trend ($P = 0.10$) for ABR.

Conclusion: Though the multivariate model suggests a trend of less bleeds with aging, descriptive results showed also higher mean ABR and JBR in oldest age category ($>40y$). This could be explained by less rFVIII-FS consumption in older PWA or a higher risk for specific bleeds in older age.

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PO275-WED

Molecular genetics and inhibitor development in patients with hemophilia B

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Background: Inhibitor in hemophilia B patients is not commonly found.

Aims: Gene mutation and other factors influencing the risk of inhibitor development in Thai hemophilia B patients were studied.

Methods: 36 unrelated hemophilia B patients were enrolled. Their phenotype and genotype were determined. The inhibitor was screened every 1–3 months during the initial 50 exposure days and then biannually or clinically indicated.

Results: Patients were classified as severe (n = 22), moderate (n = 11) and mild (n = 3) degrees. The factor IX gene mutations responsible for hemophilia B were demonstrated in every patient including missense (n = 24), nonsense (n = 4), small deletion/insertion of < 5 bp (n = 3), splice site (n = 3), and promoter (n = 2). Six of them were novel. Unfortunately, the inhibitor was found in one severe hemophilia B patient whose factor IX gene mutation revealed a point mutation at exon 2 (g. 6460 C>T) inducing stop codon. After exposure to prothrombin complex concentrate for 14 days when aged 9–12 months, he developed a high inhibitor titer of 70 BU at 12 months of age and subsequently developed anaphylaxis to factor IX concentrate at 15 months of age. In this case series, there was another severe hemophilia B patient who possessed the same factor IX gene mutation but had no inhibitor. The reported risk factors for hemophilia A patients with inhibitor including HLA class II DRB 1*15, polymorphisms at -308 of tumor necrosis factor (TNF)-alpha and -1082 of interleukin (IL)-10 were determined in these two patients with similar factor IX gene mutation. The results revealed that both patients did not possess HLA DRB 1*15, but the patient with inhibitor was heterozygote for GA at TNF-alpha and AG for IL-10 while another patient was homozygote for the wild type of GG for TNF-alpha and AA for IL-10.

Conclusion: Apart from factor IX gene mutation, TNF-alpha -308A and IL-10-1082G alleles showed the contribution to inhibitor development in one Thai severe hemophilia B patient.

Disclosure of Interest: None Declared.

PO276-WED

Haemophilia with inhibitors, update from Algiers experience, about one center

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Background: in recent years haemophilia with inhibitors is better taken care in algeria, patients are registered in a national database, two bypassing agents are currently available, the activated prothrombin complex (aPCC) and recombinant activated factor seven (rFVIIa)

Aims: the treatment of bleeding episodes in patients with inhibitors must be prepared to take a number of steps, they need to have both products available to treat bleeding episodes early in inhibitor patients, switching product early

Methods: 1843 patients were registered with the national Database (4%) of those withinhibitors, 300 patients were attending in Algiers center (10%) with inhibitors.

Results: Potential risk factors for inhibitors formation are reported: family history, age, age at first exposure, exposure days, early prophylaxis, type of concentrate, surgery. inhibitors treatment involves the control and prevention of bleeds. the dose regimen controls mild to moderate bleeding episodes utilizes a dose of rFVIIa 90 µg kg⁻¹ every 2–3 h or single dose of 270 µg kg⁻¹ a single dose of rFVIIa 90 µg kg⁻¹ was effective. or a dose of aPCC 50-80UI/kg every 12 h, protocols using rFVIIa with switch to aPCC have been used successfully in a few cases. both rFVIIa and aPCC have been used to cover surgery, the dose and duration of use for various types of surgery is not standardized, the choice of bypassing agent for a particular individual requiring surgery depends on the response of the patient to bypassing agents for previous bleed treatment. prophylaxis with bypassing agent (rFVIIa, aPCC) started in 7 children and 3 adults.

Conclusion: the lack of biological test generation of thrombin remains the main issue for patients with inhibitors follow-up and bypassing agent choice (rFVIIa or aPCC)

Disclosure of Interest: None Declared.

PO277-WED

Prevalence and risk factors associated with hypertension in hemophilia

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Background: The lifespan of individuals with hemophilia is approaching that of the general population due to safer factor products and antiviral therapy for HIV and HCV. Yet hypertension (HBP) is increasingly recognized in individuals with hemophilia (HEM), based on small uncontrolled studies. HBP is a risk factor for atherosclerotic heart disease (ASHD), and together with dyslipidemia and insulin resistance contributes to metabolic syndrome, which complicates HIV infection. Whether HBP is a complication of HEM via hematuria from kidney bleeds, proteinuria from factor infusions, or transfusion-associated HIV and HCV, is unknown.

Aims: The aim was to determine prevalence and risk factors for HBP in HEM.

Methods: We evaluated discharge data, 2009-2011, from the Nationwide Inpatient Sample (NIS) of the Healthcare Cost and Utilization Project (HCUP), which collects a 20% sample of U.S. inpatient discharges. Discharges among adult males with and without hemophilia were evaluated by age, race, year, and comorbidities. The odds ratio for HBP was estimated by univariate and multivariate logistic regression.

Results: The prevalence of HBP in HEM patients (N = 3,637), was 39.6%, significantly lower than in non-HEM patients (N = 8,025,025), 56.4%, $P < 0.001$. Associated comorbidities were also less common in HEM, ASHD, 21.4% vs 37.6%, hyperlipidemia, 25.8% vs 43.8%, and diabetes, 29.9% vs 37.7%, all $P < 0.001$. Compared to HEM without HBP, HEM with HBP were older, 60 vs 42 years, more likely to have ASHD, diabetes, and hyperlipidemia (all $P < 0.001$), but not hematuria ($P = 0.453$), proteinuria (0.149), HIV or HCV, each $P < 0.001$. The odds of HBP in HEM was significantly lower than non-HEM, OR=0.50 (CI 0.47-0.54), $P < 0.001$, and after

adjustment for ASHD, diabetes, hyperlipidemia, OR=0.92 (0.85-0.99), $P < 0.020$.

Conclusion: The risk of HBP is significantly lower in HEM than non-HEM patients, associated with lower risk of ASHD, hyperlipidemia, and diabetes. Prospective studies are warranted.

Disclosure of Interest: None Declared.

PO278-WED

A case of acute cerebral infarction in a patient with hemophilia B

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Background: Hemophilia A or B is a disease characterized by hemostatic abnormalities. As the hemophilia patients grow older, the geriatric diseases such as hypertension, diabetes mellitus or cardiovascular diseases may develop.

Aims: Cerebral hemorrhage is one of the common cause of death in hemophiliacs. But cerebral infarction is rare. We report a case of hemophilia B patient suffering from acute cerebral infarction.

Methods: 58 year old male with hemophilia B visited our hospital for right hand motor weakness. The brain MR imaging showed acute cerebral infarction in left middle cerebral artery territory. We managed him with antiplatelet drugs cautiously, giving the recombinant coagulation factor 9, Benefix, within the limits of the Korean health insurance.

Results: The patient's symptom was improved and the complications such as hemorrhagic transformation or organ/tissue bleeding did not occur.

Conclusion: When cerebrovascular accidents like infarction develop in hemophiliacs, the balance between antiplatelet drugs and coagulation factor may be important.

Disclosure of Interest: None Declared.

PO279-WED

The use of secondary prophylaxis in the haemophilia A patients

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Background: Recurrent joint bleeding leading to progressive musculoskeletal damage (hemophilic arthropathy), in spite of on-demand replacement with deficient factor concentrates, is the clinical hallmark of severe hemophilia A. Prophylactic treatment for severe haemophilia A is likely to be more effective than treatment when bleeding occurs, however prophylaxis is costly. Whereas the benefits of primary prophylaxis are well documented, data related to secondary prophylaxis are limited.

Aims: Our aim is to share our data regarding to secondary prophylaxis and to point out the benefits of secondary prophylaxis.

Methods: We are presenting the data of 115 male with the diagnosis of haemophilia A, aged between 11–338 months. Patients were grouped in to four according to bleeding frequencies. The target joint involvements were recorded. Patients response to prophylaxis treatment were also recorded by comparing the frequencies of bleedings before and after prophylaxis by chi-square test.

Results: Of all the patients, 42 were under secondary, three were under primary prophylaxis. By secondary prophylaxis bleeding episodes decreased by 78%. Nine had no change in bleeding (16%). By comparison of bleeding frequencies before and after prophylaxis by chi-square test, it was shown that the difference was statistically significant

($P < 0.01$). Three patients under primary prophylaxis had no significant bleeding.

Conclusion: Secondary prophylaxis is useful in controlling bleeding and preventing progression of joint destruction in hemophilia A patients. Failure of prophylaxis may be related to underlying status of the joints, poor compliance and participation in high-risk activities. The cost of secondary prophylaxis in adolescents and young adults is likely to be higher, as larger doses of factor concentrate are required because of patient weight, but the potential benefits of reduced absence from school, increased work based productivity and reduced need for orthopaedic surgery may be significant.

Disclosure of Interest: None Declared.

PO280-WED

Comparative study of the prevalence of clotting factor deficiency in carriers of hemophilia A and B

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Background: Hemophilia carriers are characterized by a wide range of clotting factor activity and heterogeneity of bleeding phenotype. The plasma concentrations of factor VIII (FVIII) in hemophilia A (HA) and factor IX (FIX) in hemophilia B (HB) carriers are determined by the process not entirely understood. Additionally, it is unknown whether there is a difference between values of clotting factor levels in HA and HB carriers.

Aims: The aim of this study was to compare the prevalence of clotting factor deficiency between carriers of HA and HB.

Methods: We conducted a single center study comprising all HA and HB carriers defined by genetic mutation. Additional data collected included FVIII or FIX levels and affiliation to families. Clotting factor deficiency was defined as FVIII or FIX levels < 0.50 IU mL⁻¹.

Results: In our center, 123 carriers of HA and 48 carriers of HB met inclusion criteria. The median level of FVIII in HA carriers and FXI in HB carriers was 0.61 and 0.59 IU mL⁻¹, respectively ($P = 0.69$). However, a slightly higher prevalence of clotting factor deficiency was found in carriers of HB (22 patients, 46%) then of HA (42 patients, 34%). Moreover, the factor deficiency was found in 34%, 33% and 31% of patients carrying severe, moderate and mild HA, respectively, compared to 46%, 50% and 36% of patients carrying severe, moderate and mild HB, respectively.

Conclusion: This study demonstrates higher prevalence of clotting factor deficiency among carriers of HB then HA. Besides, our results indicate that carriers of severe and moderate HB have higher risk of having factor deficiency then carriers of severe and moderate HA. The underlying mechanism of this phenomenon is unknown. Furthermore, this finding has the impact on the management of HB carriers, since they, unlike carriers of HA who can benefit from treatment with desmopressin, depend on the replacement therapy with factor IX concentrates.

Disclosure of Interest: None Declared.

PO281-WED

Prevalence and incidence of hemophilia in the North of Tunisia

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Background: Hemophilia is a hereditary genetic bleeding disorder. Its prevalence and its incidence, estimated by the World Federation of

Hemophilia, are respectively 133 cases per 1 million of male population and 1 case per 5000 male birth.

Aims: This study is aiming to estimate the prevalence and incidence of hemophilia in the north of Tunisia and compare these results to those reported in the literature.

Methods: Data are taken from the local registry of bleeding disorders, which is updated every year and the national institute of statistics. The latest census data are from 2011.

Results: According to our data, the prevalence rates are:

- 1 Hemophilia A: 67 cases per 1 million male population.
- 2 Hemophilia B: 16 cases per 1 million male population.
- 3 Hemophilia A and B: 84 cases per 1 million male population.

The incidence rates are:

- 1 in 2011: 1.3 cases per 5000 male birth
- 2 the average incidence rate during the last 5 years is: 0.8 case per 5000 male birth.

And 9 cases of death were recorded during the last 10 years.

Conclusion: The regular update of the local registry of bleeding disorders since 2005 allowed us to have objective results. The distribution of hemophilia A compared to hemophilia B in Tunisia is close enough to the ratio provided in the literature which is 5 cases of hemophilia A per 1 case of hemophilia B. The incidence rates are also close to those reported by the WFH. However the prevalence rates are low compared to those estimated by the WFH which can be explained by the existence of undiagnosed cases of hemophilia and the early death from lack of treatment some years ago.

We are recording a gradual increase of prevalence rates because of the improving of diagnosis and treatment during the recent years, and we are looking for rates of prevalence and incidence similar to those estimated by the WFH in the coming years.

Disclosure of Interest: None Declared.

PO282-WED

Effects of moderate-intensity physical exercise on pharmacokinetics of factor VIII and von Willebrand factor in young adults with severe haemophilia A

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Background: In persons with severe haemophilia A (pwshA), half-life of infused factor VIII (FVIII) can vary according to blood group, age and in response to bleeding or surgery. Exercise is recommended for pwshA, yet FVIII pharmacokinetics (PK) has not been well studied in these subjects during exercise.

Aims: To investigate FVIII PK in pwshA, in response to moderate-intensity aerobic exercise.

Methods: All subjects gave informed consent. The study was approved by a research ethic committee. Twelve young-adult pwshA with the intron-22 inversion mutation, on relatively low-dose FVIII prophylaxis regimens, and relatively good musculoskeletal status were recruited. Abbreviated PK of FVIII activity and von Willebrand Factor antigen (VWF:Ag) level were compared - during rest, and in response to 60 min exercise (two × 15 min each of stationary cycling and treadmill walking) performed at a moderate-intensity level. During rest and exercise visits, baseline blood specimens were drawn, routine prophylaxis FVIII infused, then six blood specimens were taken over the following 24 h.

Results: VWF:Ag rose transiently in all subjects post-exercise ($P < 0.01$), particularly in non-O blood group subjects, but this did not significantly alter half-life of infused FVIII (non-O blood group: 680 ± 195 vs. 646 ± 173 min at rest; $n = 5$; $P = 0.6250$. O blood

group: 503 ± 160 vs. 591 ± 166 min at rest; $n = 7$; $P = 0.1562$). No musculoskeletal bleeds occurred during the study.

Conclusion: Four × 15 min moderate-intensity aerobic exercise showed no evidence of accelerated FVIII PK or of musculoskeletal bleeding in these young-adult pwshA with relatively good musculoskeletal status, on relatively low-dose FVIII prophylaxis regimens. However, O blood group impact would merit larger studies.

Disclosure of Interest: None Declared.

PO283-WED

Prospective study on the risk, incidence and management of cardiovascular disease in older patients with haemophilia: the advance study

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Background: Persons with haemophilia (PWH) are living longer and appear to display risk factors for cardiovascular disease (CVD) with a similar prevalence to age-matched controls. SMR data for ischaemic heart disease (0.62) suggests PWH may be protected against CVD, but a cross-sectional study by the ADVANCE group showed that CVD was already present in severe (6.8%) as well as mild (12.9%) PWH aged 40–98 years. Prospective data on the incidence and management of CVD in PWH is lacking; guidance generally reflects 'expert opinion'.

Aims: The ADVANCE group, a collaboration of 20 European centres representing 6500 PWH, will collect prospective data on the incidence and management of CVD in older PWH and compare the number of observed fatal and non-fatal CVD events with events based on CVD risk prediction scores at baseline.

Methods: The prospective, observational, multi-center ADVANCE Study will run over 4–10 years on a representative sample of PWH A and B aged ≥ 40 years. The primary outcome measure will be CVD events and their management. Secondary outcomes will include other comorbidities, haemophilia status and its treatment. Ethics approval will be sought for each site and informed consent by patients is required. The ADVANCE Study is an Investigator Initiated Study funded by Bayer Healthcare.

Results: 800 patients from around 20 European centres will be recruited and followed up annually. The study will have 80% power to detect a significant difference in 10-year CVD event rate if the observed:expected event rate is <0.64 . Actual CVD event rates will also be compared with age-adjusted rates from the non-haemophilia population available from contemporary data sources. First EC approvals were achieved in Q1 2015. The study will run initially until end of 2018, when first results will be available.

Conclusion: This study will clarify CVD risk in PWH and provide evidence on which to base future preventive and treatment strategies.

Disclosure of Interest: R. C. Tait Consultant for: Bayer HealthCare, Speaker Bureau of: Bayer HealthCare, P. A. Holme Consultant for: Bayer HealthCare, Speaker Bureau of: Bayer HealthCare, C. Combes: None Declared, P. de Moerloose: None Declared, E. Berntop: None Declared, R. Schutgens: None Declared, R. Klamroth Consultant for: Bayer HealthCare, C. Hermans: None Declared, S. Rauchensteiner Employee of: Bayer HealthCare, G. Dolan: None Declared.

PO284-WED

Longevity of non-pathogenic antibodies against human recombinant proteins in healthy individuals

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Background: The origin and biological significance of non-pathogenic antibodies against self-proteins, as seen in some healthy individuals, remains a matter of debate. Cohen (J Autoimmun 2007) suggested that auto-antibodies present in healthy individuals are biomarkers of the immunological homunculus, the body's system for sensing the current immunogenic state and ensuring immune homeostasis. Recently, we demonstrated that some healthy individuals express low-titer antibodies against human recombinant proteins, including FVIIa, FVIII, FIX and VWF, which resemble self-reactive antibodies.

Aims: To assess the longevity of non-pathogenic antibodies against human recombinant proteins in healthy individuals

Methods: ELISA assays to detect specific antibodies against human recombinant FVIIa, FVIII, FIX and VWF were established in compliance with regulatory guidelines. Cohorts of 480–600 healthy individuals were screened for the presence of antibodies followed by determination of antibody titers and confirmation of antibody specificities. For longitudinal observations, 38 healthy individuals were followed for 14 months with blood samples taken every month.

Results: Our data confirm the presence of antibodies against human recombinant proteins (FVIIa, FVIII, FIX, VWF) in a proportion of healthy individuals, with a prevalence ranging from 1.25% to 19%. Antibody titers were mostly below 1:80 (1:20, 1:40), but up to 1:640 in some samples. The presence of antibodies against one protein did not predict the presence of antibodies against another protein in the same sample. Longitudinal studies indicated that some individuals had antibodies that persisted for the whole observation period.

Conclusion: Non-pathogenic antibodies against a range of human recombinant proteins are found in healthy individuals. These antibodies are persistent and resemble non-pathogenic self-reactive autoantibodies. Their biological significance remains to be shown.

Disclosure of Interest: F. Horling Employee of: Baxter BioScience, C. Hofbauer Employee of: Baxter BioScience, P. Allacher Employee of: IMC Fachhochschule Krems, S. Till Employee of: Baxter BioScience, B. Abbuehl Employee of: Baxter BioScience, F. Scheifflinger Employee of: Baxter BioScience, B. Reipert Employee of: Baxter BioScience.

PO285-WED

Plasma protein abundances of proteins involved in coagulation and bone/cartilage turnover are altered after haemophilic joint bleed

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Background: Haemophilia A is a bleeding disorder where the blood does not clot normally. An untreated bleed may cause severe symptoms, and bleeding into a joint may lead to development of haemophilic arthropathy. Current evaluation of a bleed is subjective and based on unspecific clinical symptoms. A biomarker panel would provide an objective clinical tool for optimized treatment and an objective outcome in clinical trials aiming at prevention of bleeding.

Aims: The aim of this project was to identify potential biomarkers of bleeding in a haemophilic rat model.

Methods: Recently, a FVIII-/- rat was generated as a translational model of human haemophilia (Nielsen et al. (2014) JTH). In this study controlled haemarthrosis was induced by needle puncture through the infra-patellar ligament of the left knee in the FVIII-/- rat (n = 10). Citrate stabilised (CS) whole blood was obtained from the tail vein before

injury (control) and at 2, 6 and 24 h post injury. B-mode ultrasonography at 24 h post injury confirmed blood within the joint cavities. The blood samples were centrifuged (4000 g for 5 min) and the CS-plasma was obtained and frozen at -80°C until analysis. High resolution mass spectrometry and iTRAQ® strategy was used to identify proteins with altered plasma concentrations at the different time points compared to controls. A combinatorial workflow of pre-fractionation techniques was designed in order to overcome the high dynamic range of plasma protein abundances. Principal Component Analysis (PCA) and ANOVA analysis was carried out.

Results: Approximately 400 proteins were identified and quantified by this combinatorial workflow, estimated to cover protein abundances within 6–7 orders of magnitude.

Conclusion: PCA identified the protein composition at the 24 h time-point to differ the most from the control group. ANOVA analysis identified 35 proteins regulated post-bleed (*P*-values < 0.05). Among the regulated proteins were proteins known to be involved in coagulation and bone/cartilage turnover.

Disclosure of Interest: I. Rodríguez León Employee of: Novo Nordisk A/S, M. Enoksson Employee of: Novo Nordisk A/S, L. Nikolic Nielsen Employee of: Novo Nordisk A/S, K. Roepstorff Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, M. Kjelgaard-Hansen Employee of: Novo Nordisk A/S, E. Norling Olsen Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, B. Vandahl Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S.

PO286-WED

Immune tolerance induction experience with advate: final results of an international prospective ITI registry (PAIR)

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Background: The Prospective ADVATE Immune Tolerance Induction Registry (PAIR) is designed to collect information on ADVATE safety and effectiveness in ITI therapy in routine practice.

Aims: To collect information on ADVATE safety and effectiveness in ITI therapy.

Methods: The study enrolled individuals with hemophilia A and inhibitors from 10 countries. Primary objective: to assess the incidence of adverse events (AEs) related to ADVATE during ITI therapy. Secondary objectives: incidence of central venous access device (CVAD)-related complications, and success rates of ITI therapy. Maximum observation period for ITI is 33 months plus a 12 month follow-up.

Results: As of Nov. 6, 2014, 36 of 44 subjects (81.8%) completed ADVATE ITI therapy; 6 subjects withdrew prior to completion; 28 (63.6%) completed the 12 month follow-up. The most commonly prescribed regimen was 90–130 IU kg⁻¹ day⁻¹ (n = 26, 59.1%). During the observation period, a total of 337 bleeding episodes and 280 AEs were reported (N = 44). Fifty-five AEs (19.6%) were serious and none were considered related; 14 AEs (5.0%) were non-serious and related. Thirty-two subjects experienced one or more CVAD-related AE /complications. Of subjects completing ITI, 21 achieved negative inhibitor titer, two experienced a high to low titer conversion, eight failed to achieve negative titer, and five were un-assessable per protocol. After 18 months therapy, Kaplan-Meier estimates of success for achievement of first negative titer was 68.3% (asymptotic 95% CI: 51.8–83.6%, n = 36) for the completer's group. Rates were higher for the per protocol analysis set (72.4%, CI: 55.5–87.0%, n = 31), and slightly

lower for the full analysis set (65.8%, CI: 50.5-80.5%, n = 44). All subjects have now exited the study; final results will be presented.

Conclusion: These late-stage interim results are consistent with previous reports on ADVATE in ITI. No new related safety issues have been seen and ITI was effective in most. Final database analysis will add knowledge on prognosticators of ITI success.

Disclosure of Interest: A. Shapiro: None Declared, K. Khair: None Declared, J. Teitell: None Declared, I. Leony-Lasso: None Declared, K. Steinitz Employee of: Baxter Healthcare Corporation, A. Gringeri Employee of: Baxter Healthcare Corporation, G. Spotts Employee of: Baxter Healthcare Corporation.

PO287-WED

Frequency of bleeding events in patients with hemophilia on prophylaxis based on a commercial insurance claims database

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Background: Real world data lack treatment outcomes in patients with hemophilia on continuous prophylaxis.

Aims: To quantify bleeding events in patients described above using real world data.

Methods: We used 2004-2012 Truven MarketScan database, which has administrative claims of insurance payment requests for healthcare services. We included males < 65 years with hemophilia A (HA; ICD9: 286.0) or B (HB; 286.1) without inhibitors, and ≥ 12 months continuous benefit enrollment from the first hemophilia treatment observed. Bleeding events were identified from inpatient/outpatient claims using ICD9 codes for hemarthrosis, hematoma or other acute bleed. The World Federation of Hemophilia (WFH) recommends treating a typical bleed for 1-7 days. Thus, a bleeding event was defined as a cluster of bleeding claims within a 7-day span. At patient-year level, we identified prophylaxis use by categorizing total number of clotting factor-related prescription (Rx) claims supplied for > 45 weeks year⁻¹ (WFH definition), assuming 2-3/week infusions for HA and 1-2/week infusions for HB patients. Since episodic treatment for frequent bleeding may mimic prophylaxis usage, to reduce potential misclassification, we removed Rx used for treating bleeds and excluded patients with yearly supply above our defined upper limit for prophylaxis use.

Results: We identified 303 person-years among HA (13.5% of total) and 21 person-years among HB patients (11.0% of total) receiving prophylactic treatment. Among HA prophylaxis group, average annual bleed was 0.8 (median: 0, range:0-13). In similar HB group, average annual bleed was 0.2 (median: 0, range:0-1).

Conclusion: This is the first study to quantify hemophilia bleeds for prophylaxis patients using claims. The bleeding frequencies observed lie towards the lower end of the range observed for such patients in clinical trials. As many patients manage their bleeds at home, bleeding rates calculated based on health resource utilization may underreport the true rate.

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PO288-WED

Improvement of the quality of health care for children with severe haemophilia A using prophylaxis and treatment of bleeding episodes with recombinant factor VIII

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Background: Prophylaxis is a very important development in the lives of children with severe haemophilia. Primary prophylaxis with regular factor VIII infusions started at an early age of life is recommended as the optimal care for children with severe haemophilia A.

Aims: The objective of this study is to evaluate safety, efficacy and benefits of replacement therapy with recombinant factor VIII (rFVIII) - turoctocog alfa for prophylaxis and treatment of bleeding episodes in 5 young children (1-7 years).

Methods: The previously treated children with ≥ 50 exposure days to any FVIII product and no history of inhibitors received prophylaxis with rFVIII (30 IU kg⁻¹ three times weekly for 4 children, and 30 IU kg⁻¹ every second day for one child). Mean exposure to rFVIII was 60 exposure days per patient.

Results: None of the patients developed inhibitors (≥0.6BU). Parents of patients were educated to give rFVIII at home and to register every prophylactic dose and bleeding episodes in diary. Based on patients reports the median annualized bleeding rate was 5.0 bleeds per patient per year. The success rate defined as 'excellent' or 'good' haemostatic response for treatment of bleeding episodes was 92%. The bleeding episodes (95%) were controlled with 1-2 infusions of rFVIII. No thromboembolic events or allergic type hypersensitivity reactions occurred during the treatment. According to the questionnaire filled in by parents about quality of life of children treated with rFVIII for prophylaxis and treatment of bleeding episodes, children had rare bleeds; they regularly visited kindergarten and school, and had a normal active lifestyle.

Conclusion: Results from the presented study showed that prophylaxis and treatment of bleeding episodes with rFVIII in children with severe haemophilia A was safe, effective and improved quality of life of patients.

Disclosure of Interest: None Declared.

PO289-WED

Inhibitor development as switching full-length recombinant FVIII to B-domain deleted recombinant FVIII in previously treated patients with hemophilia A: Taiwan's experience

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Background: The previous studies revealed that no increased risk of inhibitor development was seen in patients with hemophilia (PWH) switching from full-length rFVIII (FL-rFVIII) to B-domain deleted rFVIII. Most of these studies came from West countries, and experience from Asian countries was much less.

Aims: We aim to report our experience of switching to Moroctocog alfa (AF-CC) in previously treated patients (PTPs) with hemophilia A in Taiwan.

Methods: As AF-CC had been used in Taiwan since 2012, PTPs who experienced treatment of AF-CC switching from FL-rFVIII in Shuang Ho Hospital from 2012 to Dec. 2014 were enrolled. Data of age, severity, and inhibitor status were collected and analyzed.

Results: There were totally 8 Taiwanese PTPs with hemophilia A (7 severe type, one moderate type), with mean age of 40, ranging 22 from 67. Of the 8 PTP, 7 received Advate® and one received Kogenate-FS® before switching, without the history of inhibitor development. After switching to AF-CC, all the 8 PWH received on-demand therapy. During the study time, 4 PWH were found to have inhibitors. Three severe-type PWH developed transient low-titer inhibitors with titers of 0.83, 0.74, and 0.94BU, which occurred on exposure days (ED) of 30, 91, 145, respectively. Their inhibitors became undetectable rapidly at following monitors. Another was a 25-year-old severe-type PWH, whose gene defect was c.6670_6672delCCT. He switched from Kogenate-FS® and found to develop inhibitors with titer of 6.3BU on 44 ED and 14BU on 58 ED. Immune tolerance induction (ITI) therapy was started with Kogenate-FS® 50 IU kg⁻¹ 3 times week⁻¹. His inhibitor titer became < 0.5BU 4 months later and successful ITI therapy was achieved next 2 months later.

Conclusion: Our study showed the experience of AF-CC in Taiwanese PTPs. Most inhibitors developed transiently with low titers. One PWH with high-titer inhibitors had successful ITI therapy. It needs the further prospective large studies to clarify the prevalence of inhibitors in Asian PTPs after switching to AF-CC.

Disclosure of Interest: None Declared.

PO290-WED

Ultrasonography of joints and correlation with function in haemophilic arthropathy – results of a clinical pilot trial (hämarthrosopilot) and potential importance of joint-ultrasound for an individualized prophylaxis

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Background: Sonography is applied in patients with bleeding disorders. By ultrasonography is a sign of the activity of an existing hemophilic arthropathy the extent of synovitis, as a sign of progression are defects of articular cartilage and bony structures quantifiable.

Aims: The sonographic quantification of the arthropathy by haemophilia-treaters could be the basis for better individualized haemophilia therapy in young patients.

Methods: For this purpose, highly reproducible sonographic standard section planes and an ultrasound score (HEAD -US) were published by C. Martinoli examiner-independently. After a preliminary study of 27 patients now we have included 177 young patients (children and young adults) with haemophilia A, haemophilia B or von-Willebrand-disease from German hemophilia treatment centers in the Hämarthrosopilot study (DRKS00004483).

After informed consent sonography of the elbow, knee and ankle joints with a Zonare 'z.one ultra' "ultrasound device (Linear probe L14 -5w) was performed. Simultaneously an orthopedic clinical examination and 3D motion analysis of the lower limbs were performed with an ultrasonic topometer.

Results: Already in the previous investigation of the pilot study on a small cohort yielded promising results depending on age. The investigations in the presented pilot study showed correlation of the sonographic diagnostics with the measurement of a clinical orthopedic examination in haemophilic arthropathy. Through the joint sonography changes were even partially already seen before that stood out in the clinical investigation.

Conclusion: It may be useful if haemophilia treaters in future apply this method to individualize the therapy under close control and evaluation of joint changes.

Disclosure of Interest: None Declared.

PO291-WED

Comparing cardiovascular disease in patients with hemophilia with data in the general population: methodological considerations

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Background: Publications on cardiovascular comorbidities in patients with hemophilia (PWH) have shown inconsistent results. The ADVANCE group has collected cross-sectional data on comorbidities in PWH aged ≥ 40 years. Unbiased, comparative epidemiological data in the non-hemophilia population is needed for valid comparisons.

Aims: The ADVANCE group commissioned a review of cardiovascular disease (CVD) data in national general population studies.

Methods: Structured (EMBASE and MEDLINE) and 'grey' literature searches identified relevant European data sources, which were then screened for inclusion of patient-level data on CVD risk factors, events in age groups > 40 years. Sources were excluded if data were unpublished or inaccessible, sample size was small (< 500 men) or had no age stratification or included only mortality data. Identified sources were prioritized according to representativeness, accessibility and granularity of data for appropriate comparisons.

Results: For Germany, one national study was identified. Rates of CVD in men aged ≥ 40 years were 10.8–14.0% for coronary heart disease (Göbwald 2013) and 2.6–4.2% for stroke (Busch 2013). Rates of hypertension were 37.3–46.2% in 50–59 year-old and 53.5–63.2% in 60–69 year-old men (Neuhauser 2013). In the ADVANCE H3 cross-sectional study, the rate of coronary artery disease was 5.6% and stroke was 2.6%. Hypertension rates were 51% (50–60 years) and 57.3% (60–70 years).

Conclusion: Comparison of hypertension rates in different populations is vague due to possible under-diagnosis in the general population; comparisons based on CVD events may be more objective. For unbiased comparisons, definitions of subpopulations with comorbidities should be identical. Only access to existing databases offers the chance to accurately match results from studies of PWH with the general population. Ideally, comparisons should be made with sources from within each target country.

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PO292-WED

Bleeding events in Chinese children with severe hemophilia A receiving standard prophylaxis vs on-demand treatment with Bayer's sucrose-formulated recombinant factor VIIIZhao Y¹, Xiao J¹, Yang R², Wu R³, Hu Y⁴, Beckmann H⁵, Wu J⁶, Hou D⁶ and Sun J⁷

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Background: In China, current standard of care for severe hemophilia is on-demand treatment and secondary low-dose prophylaxis (10 IU kg⁻¹ 2 × wk⁻¹). This is the first prospective, standard-dose, tertiary prophylaxis study in China.

Aims: To evaluate 3x/wk prophylaxis with Bayer's sucrose-formulated recombinant factor VIII (rFVIII-FS), approved for pediatric prophylaxis in China, vs on-demand treatment in Chinese children with severe hemophilia A

Methods: This 24-week, sequential-treatment study included previously treated males aged 2–16 years with severe hemophilia A, no inhibitors, and no prophylaxis for > 6 months prestudy. Patients were treated on demand for 12 weeks, then switched to 12 weeks of prophylaxis (rFVIII-FS 25 IU kg⁻¹ 3 × wk⁻¹). Efficacy variables included annualized number of all bleeds (primary variable), joint bleeds, health outcomes (school attendance/activity and daily activity), number of infusions to control bleeds, and patient assessment of treatment response.

Results: For the 30 patients (median age 12 years), median (range) rFVIII-FS prophylaxis dose was 27.1 (22–45) IU/kg/infusion. Compared with on demand, prophylaxis reduced median (quartile [Q]I; Q3) annualized number of all bleeds (57.5 [45; 74] vs 0 [0; 4]) and joint bleeds (34.5 [26; 57] vs 0 [0; 4]). Mean school attendance/activity and daily activity improved with prophylaxis vs on demand. 19 patients (63.3%) on prophylaxis vs 0 patients treated on demand were bleed-free, indicating good bleeding control with prophylaxis. During the on-demand and prophylaxis phases, 89.1% and 86.4% of bleeds, respectively, were treated with ≤ 2 infusions. Patient assessment of treatment response was good or excellent for ≥94% of all bleeds. No inhibitors, treatment-related adverse events (AEs), or serious AEs were reported.

Conclusion: In this first standard prophylaxis study in China, rFVIII-FS prophylaxis reduced bleeding events and improved health outcomes vs on-demand treatment in children with severe hemophilia A.

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PO293-WED

Prophylactic efficacy of twice-weekly vs. 3-times-weekly bay 81-8973 in severe hemophilia A: results of the Leopold I and II clinical trialsMahlangu JN¹, Kavakli K², Hvitfeldt Poulsen L³, Tseneklidou-Stoeter D⁴, Beckmann H⁵, Enriquez MM⁵ and Rusen L⁶

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Background: Prophylaxis regimens for hemophilia A require infusions 2x/wk or 3x/wk, and frequency of infusion is a potential barrier to adoption of prophylaxis. BAY 81-8973 is a new full-length recombinant factor VIII (FVIII) manufactured with technologies to optimize clinical performance.

Aims: To compare efficacy of 2x/wk vs 3x/wk prophylaxis with BAY 81-8973 in the LEOPOLD I and II trials

Methods: Key eligibility criteria in LEOPOLD I and II were age 12–65 years, severe hemophilia A, ≥ 150 exposure days to any FVIII product, and no inhibitors. BAY 81-8973 prophylaxis was given at 20–50 IU kg⁻¹ 2 or 3 times week⁻¹ in both trials. In LEOPOLD I, dose and dosing regimens were assigned by the investigator (81% of patients received prior prophylaxis); in LEOPOLD II, patients previously treated on demand were randomized to the treatment regimens. The primary efficacy endpoint for both trials was annualized bleeding rate (ABR) for all bleeds. Prophylaxis with BAY 81-8973 2x/wk vs 3x/wk was analyzed and compared in the trials.

Results: In LEOPOLD I, 18 vs 44 patients received 2x/wk vs 3x/wk prophylaxis with BAY 81-8973 (LEOPOLD II, 28 vs 31 patients). Median (mean) ABR for patients in the 2x/wk vs 3x/wk dosing regimens was 1.0 (4.8) vs 2.0 (3.4) in LEOPOLD I and 4.0 (5.7) vs 2.0 (4.3) in LEOPOLD II (first 6 months of LEOPOLD II: 4.1 [7.7] vs 2.0 [4.5]; second 6 months: 1.1 [3.7] vs 2.0 [4.1]). In the 1-year treatment period, 16 patients remained bleed-free during both LEOPOLD I (2x/wk, n = 6 [33.3%]; 3x/wk, n = 10 [22.7%]) and LEOPOLD II (2x/wk, n = 8 [28.6%]; 3x/wk, n = 8 [25.8%]).

Conclusion: In LEOPOLD I and II, 2x/wk and 3x/wk BAY 81-8973 prophylactic dosing regimens were both efficacious in achieving bleeding control despite differences in treatment assignment in the 2 trials (investigator assigned vs randomized). These results highlight efficacy of less frequent BAY 81-8973 prophylactic dosing regimens after 6 months of treatment in ≥50% of patients with no increase in bleed rate or FVIII consumption.

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PO294-WED

Expanding communications on hemophilia A outcomes (ECHO) study: a longitudinal, observational disease registry of patient-reported and clinical outcomes in patients with hemophilia A

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Background: Wide variations in treatment patterns and the emergence of new therapeutic options for patients with hemophilia A are associated with limited knowledge of the link between clinical and patient-reported outcomes (PROs) and treatment patterns.

Aims: The Expanding Communications on Hemophilia A Outcomes (ECHO) study, a 5-year, international, prospective, observational registry for adult male patients with moderate to severe hemophilia A, was initiated to address this knowledge gap.

Methods: 2000 patients will be enrolled from 9 countries in Western Europe, North America, and Asia and will be followed for at least 2–5 years. Data will be collected at baseline and every 12 months thereafter. Clinical data will be collected by chart review and electronic patient diaries. PRO instruments include the EuroQoL 5-Dimension questionnaire (EQ5D), Haemophilia Activities List (HAL), Work Productivity and Activity Impairment Scale (WPAI), 12-Item Short-Form Health Survey (SF-12), Hemophilia Treatment Satisfaction Questionnaire (HEMO-SAT), Validated Hemophilia Regimen Treatment Adherence Scale (VERITAS PRO/PRN), and Brief Pain Inventory (BPI). Patients will complete a bleeding diary (ie, Haemtrack or similar system) weekly for the first year and for ≥6 months following a major treatment change. Haemtrack has been implemented at all UK hemophilia centers and shown to be a useful tool to optimize treatment. Statistical analysis of the data will be descriptive in nature.

Results: Data collection will begin in 2015, with the first analysis planned for 2016.

Conclusion: PROs are becoming increasingly important in the changing treatment landscape for hemophilia A. Forthcoming results from ECHO will describe the real-world influence of patient, treatment, and disease characteristics on patient functioning, well-being, resource utilization, and economic burden.

Disclosure of Interest: C. Hay Consultant for: Bayer HealthCare, N. Church Employee of: Bayer HealthCare, S. Choudhri Employee of: Bayer Inc, C. Kessler Grant/Research Support from: Bayer HealthCare, Consultant for: Bayer HealthCare.

PO295-WED

Effective radioactive synovectomy in hemophilia with inhibitors

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Background: Joint bleeding is the major problem in patients with hemophilia and haemophilic synovitis is still the major clinical problem. Bleeding control is more difficult in inhibitor positive patients. Radioactive synovectomy (RS) is a very effective intervention that decreases both the frequency and the intensity of recurrent joint bleeds related to synovitis.

Aims: We retrospectively reviewed our RS results in hemophilia with inhibitors.

Methods: The patients referred from the Hemophilia Society of Turkey and evaluated by hemophilic arthropathy council. The indication for RS was clinical diagnosis of synovitis, presence of 3 or more hemarthrosis into the same joint within the last 6 months and prolonged bleeding in the same joint more than two months. Radiosynovectomy was performed 52 joints (18 ankle, 17 knee, 13 elbow, 2 hip and 2 shoulder) of 20 Hemophilia A with inhibitors. The median age was 14.4 (range: 3–41) years old. Patients were followed up by clinical evaluation based on assessments of joint-bleeding frequency. In 5 patient's 9 joints (4 elbow, 3 ankle, 2 knee) RS was repeated.

Results: In once RS performed group, median follow-up was 3 years (range: 0.66–15 years) and median bleeding frequency was 3 per month (range: 1–4) before RS. A marked decrease (an 75%>100% decrease) in bleeding episodes was seen in all of the patients except one who had ankle arthrodesis. In the repeated RS group, the second RS median time was 3 (range: 1–4) years; median follow-up was 5 years (range: 0.5–10 years) and median bleeding frequency was 2 per month (range: 1–3) before RS. After repeated RS all of the patients had marked decrease in bleeding episodes except one who had knee arthroscopy. There was no any major complication except one gluteal hematoma after left hip RS.

Conclusion: Radiosynovectomy is very effective and safe procedure also in haemophilia with inhibitors. And also RAS can repeated performed 2–3 times at the same joint if the bleeding is not under control.

Disclosure of Interest: None Declared.

PO296-WED

Improving hemophilia care in Brazil: availability of factor concentrates and beyond – results of LEVANTH survey

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Background: The greater availability of factor concentrates since 2011 changed the scenario of hemophilia care in Brazil.

Aims: This survey aimed to draw the current profile of hemophilia in Brazil, in order to identify aspects that can still improve.

Methods: Professionals from 34 hemophilia treatment centers in Brazil were invited to answer a structured questionnaire about the treatment offered to hemophilia patients.

Results: 11 of 34 centers (32%) returned the questionnaire with valid answers. A total of 4468 individuals (42% of 10,923 hemophilia patients registered in 2012) from all regions in Brazil were represented by these 11 centers. Among patients with severe hemophilia A (HA) without inhibitors, 47% up to 2 years and 32% from 3 to 12 years are still treated on demand. For severe hemophilia B (HB) the values were 33% and 40%, respectively. Most patients older than 18 years with severe HA or HB are treated on demand. 47.2% of 199 patients with HA and inhibitors are under immune tolerance induction (ITI), while 52.3% had never undergone ITI. Among 182 patients with HA and inhibitors treated with bypass agents, 12% receive prophylaxis with bypass agents and ITI, but in the subgroups of patients up to 12 years (n = 69) and from 13–24 years (n = 55), 19% and 13% respectively receive prophylaxis with bypass agent, but are not under ITI. Regarding other aspects that hinder optimal treatment, social problems, lack of professionals for self-infusion training, inadequate compliance and the difficult access to the treatment center were the most cited. Only 5 centers encourage patients to keep a diary of infusions to monitor compliance. 306 patients (average 34 per center) were eligible for orthopedic procedures, but only 4 centers have an active orthopedic team involved with hemophilia.

Conclusion: LEVANTH was able to characterize current practices in hemophilia in Brazil and identified important aspects, beyond the availability of factor concentrates, to be improved.

Disclosure of Interest: None Declared.

PO297-WED

Prevalence, description, and management of pain in adult people with hemophilia (PWH): initial lessons from the pain functional impairment and quality of life (P-FIQ) study

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Background: Hemophilia is marked by frequent hemarthrosis, resulting in acute and chronic joint pain. Regional/national surveys in US adult PWH have demonstrated suboptimal strategies for pain management.

Aims: To assess pain prevalence, description, and treatment in adult PWH.

Methods: Sequential adult male PWH (mild-severe) with history of joint pain/bleeding enrolled during routine visits completed a hemophilia/pain history (described below) and various patient-reported outcome instruments (PROs).

Results: Of 164 PWH completing optional PRO retests (median age 34), more had hemophilia A (74%) than B (26%); 6% had inhibitors. The majority had some college or above education (63%) and were employed (81%); 61% were overweight or obese, and 61% self-reported arthritis/bone/joint problems. Current patient-reported treatment regimens were prophylaxis (42%), on-demand (39%), or mostly on-demand (19%, 25/31 using infusions ahead of activity). Subjects reported acute pain only (24%), chronic pain only (33%), or both (29%); only 15% reported no pain in the past 6 months. Acute pain was most frequently described as sharp (77%), aching (66%), shooting (57%), and throbbing (55%). Chronic pain was most frequently described as aching (74%), nagging (49%), throbbing (44%), and sharp (40%). Most common analgesics for acute/chronic pain were acetaminophen (69%/58%), NSAIDs (40%/52%), hydrocodone-acetaminophen (29%/33%). Most common nonpharmacologic strategies for acute/chronic pain in the past 6 months were ice (73%/37%), rest (48%/34%), factor or bypassing agent (48%/24%), elevation (34%/28%), and relaxation (31%/23%). Alcohol was reported by 8%/7% and medical marijuana by 17%/9%.

Conclusion: Initial data from P-FIQ substantiate the high prevalence of pain in adult PWH. Like the national/regional surveys, it highlights the opportunities to improve assessment of and dialogue with patients, as well as to differentiate strategies for managing acute bleed-related vs chronic arthritic pain.

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PO298-WED

Impact of pain and functional impairment in us adult people with hemophilia (PWH): results from retest population in the pain functional impairment and quality of life (P-FIQ) study

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Background: Hemophilia is marked by frequent hemarthrosis, resulting in pain and functional impairment. Standardized and disease-specific patient-reported outcome (PRO) instruments have been used in clinical studies of PWH, but rarely to individualize treatment plans.

Aims: To assess pain and functional impairment through 5 PRO instruments in nonbleeding PWH.

Methods: Sequential adult male PWH (mild-severe) with history of joint pain or bleeding enrolled during routine visits completed a pain history and 5 PROs (EQ-5D-5L with visual analog scale [VAS], Brief Pain Inventory [BPI], SF-36, International Physical Activity Questionnaire [IPAQ], and Hemophilia Activities List [HAL]). Median (Q1, Q3) PRO scores were calculated on the initial completed PRO in patients completing optional PRO retests.

Results: Of 381 patients enrolled from 10/2013 to 10/2014, 164 completed the retest (median age 34). EQ-5D VAS was 80.0 (65.0, 90.0), and EQ-5D Health Index was 0.80 (0.69, 0.86); 73.2% reported problems with pain-discomfort, 61.6% with mobility, 55.8% with usual activities, 41.1% anxiety-depression, and 22.0% problems with self-care. For BPI, pain severity and pain interference were 3.0 (1.3, 4.8) and 2.9 (0.7, 5.2), respectively; specifically, median worst pain was 6.0, least pain 2.0, average pain 3.0, and current pain 2.0. Ankles were the most frequently reported site of pain. IPAQ total activity was 693.0 (346.5, 1324.0) METs; 73 patients (49.3%) reported no activity in the prior week. SF-36 overall health score was 3.0 (2.0, 3.0); median score for physical health was 39.6 and 51.6 for mental health. Overall HAL score was 76.1 (59.2, 95.1).

Conclusion: Data from the retest population demonstrate the challenges of pain and functional impairment in adult PWH. Pain was ubiquitous, and impact on daily activities and quality of life was noted in each PRO. Ongoing analysis of the complete study population will include individual question responses and assessment of covariates.

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PO299-WED

A retrospective study of the current treatment practice of haemophilia A and B in the United Kingdom

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Background: Treatment practice of haemophilia A and B varies significantly between individuals, countries and regions and real-life bleeding and treatment data are often incomplete or lacking detail, hampering the planning of future healthcare resource allocations.

Aims: To provide a detailed account of the bleeding pattern and current FVIII/FIX treatment practice, in a real-life setting, for patients with haemophilia A or B in the United Kingdom (UK).

Methods: This is part of a larger ongoing, non-interventional, retrospective study exploring factor usage for haemophilia A and B in several European countries. Anonymized UK data from Apr 2012-Mar 2013 were retrieved from the National Haemophilia Database (NHD), which is a register for patients with bleeding disorders in the UK, and Haemtrack, an online system for patients to self-report bleeding and treatment details. Patients with basal FVIII/IX levels ≤ 5 IU dL⁻¹ and without inhibitors were included. No study-specific patient consent was required for the UK according to applicable laws. All data will be presented by descriptive statistics.

Results: In total, 1458 haemophilia A and 399 haemophilia B patients were enrolled from the NHD, and 302 and 100 from Haemtrack, respectively. The Haemtrack population was selected for further analysis on basis of data-completeness criteria. Haemtrack shows the amount of factor used, frequency and dosage of injections, annualized bleeding rates, type of bleed (joint, muscle/soft tissue, mouth or other) and efficacy in treating bleeds. Data will be presented by type of haemophilia (A or B), treatment regime (prophylactic, partly prophylactic or episodic treatment), age group, severity (severe/moderate) and type of product (recombinant/plasma derived). Complete data are presently being analyzed and will be presented at the congress.

Conclusion: These data will provide an updated view on the real-life bleeding patterns and the current individual treatment practice for patients with haemophilia A or B in the UK.

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PO300-WED

Immune tolerance induction in patients with severe hemophilia A with inhibitors in Korea

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Background: Inhibitors to factor VIII(FVIII) are important complications when managing patients with hemophilia A. Immune tolerance induction(ITI) has been thought to be the useful method for the eradication of inhibitors.

Aims: We report the results of a retrospective study in hemophilia A patients who underwent ITI in Korea.

Methods: A retrospective chart review was conducted from March 2004 to November 2013 at Korea hemophilia foundation clinic. ITI started at the dose of FVIII concentrates 100 U kg⁻¹, three times a week. According to inhibitor titer and FVIII recovery rate, dose of FVIII concentrates was reduced. The inhibitor elimination was defined as the time taken to achieve a negative inhibitor assay with no anamnestic response and normal FVIII recovery and/or normal half-life.

Results: Seventeen patients with severe hemophilia A were evaluated. Their median age at the start of ITI treatment was 5.8 years (range: 2.8~21.6). They were diagnosed with hemophilia A in a median age of 6.0 months and detected inhibitors at the median age of 2.0 years. The median peak inhibitor titer before ITI was 32.4 BU mL⁻¹. Median treatment duration was 25.7 months. Complete immune tolerance was achieved in 14 of 17 patients (82%). Eleven patients received von Willebrand factor-containing plasma-derived FVIII concentrates. Of these, seven(7/11, 82%) patients achieved inhibitor elimination. Success rates were higher in younger patients (≤ 7 years) and in patients treated < 5 years after inhibitor detection. Historical peak inhibitor titer and maximum inhibitor titer during ITI was also low at complete tolerance group. All tolerized patients continued to receive prophylactic therapy after ITI, with no inhibitor recurrence from the end of ITI.

Conclusion: This study shows that ITI would be the effective and well-tolerable method for the eradication of inhibitors. Influencing factors for ITI success were age at start of ITI treatment and duration after inhibitor detection.

Disclosure of Interest: None Declared.

PO301-WED

The usage of recombinant activated factor VII (rFVIIa) during major and minor surgeries in severe hemophilia patients with inhibitor

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Background: Inhibitors are the most serious complication of hemophilia therapy. Inhibitors significantly increase morbidity and also lower 'quality of life' in hemophilia patients. Treatment of inhibitors and surgeries in hemophilic patients with inhibitors are difficult and extremely expensive.

Aims: We report that our surgical experience in 9 patients with hemophilia with inhibitors.

Methods: We retrospectively reviewed medical records of 9 hemophilic patients with inhibitors who required major or minor surgery between 2001 and 2014. We used rFVIIa at the dosage of 90 µg kg⁻¹ every 2 h except one patient.

Results: During this period, 15 surgical procedures were performed in 9 hemophilia patients with inhibitors, age ranging from 5 months to 19 years with a average of 11 years. One intracerebral and subdural hematoma drainage, 5 circumcision, 1 cataract extraction, 1 port a cath insertion, 1 tonsil cauterizing and clot aspiration with bronchoscopy, 1 dental extraction and 4 radioisotope synovectomies were performed. Only one patient with intracranial bleeding died postoperative periode. There were no thromboembolic complications during rFVIIa treatment. Blood transfusion was given in 2 patients.

Conclusion: Surgical procedures in hemophilia patients with inhibitors can present a challenge because of the increased risk for bleeding complications and the potential difficulty in controlling bleeding during and after surgery. This report showed that hemophilia patients with inhibitors need comprehensive and experienced hemophilia treatment centers.

Disclosure of Interest: None Declared.

PO302-WED

Intracranial hemorrhage and management in hereditary bleeding disorders: the experience of Cukurova University, Adana, Turkey

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Background: Intracranial hemorrhage (ICH) is a life threatening complication of hereditary bleeding disorders in childhood resulting in high rates of mortality and disabling sequelae.

Aims: In this study, we evaluated our patients with intracranial hemorrhage and compared with literature.

Methods: From 1995 to 2014, 27 patients with intracranial hemorrhage were diagnosed and evaluated in Çukurova University Hemophilia Center. ICH episodes, the findings of physical examination, CT scan or MRI and treatment strategies including surgical interventions were reviewed retrospectively.

Results: We evaluated 28 episodes of ICH from 27 patients with hereditary congenital factor deficiencies (CFD). Age range was from 9 days to 12 years. There were 18 patients with hemophilia A, 2 patients with hemophilia B, 2 patients with factor VII deficiency, 4 patient with factor X deficiency, 1 patient with von Willebrand disease. Two patients with factor VII deficiency, 2 patients with factor X deficiency and 1 patient with von Willebrand disease were female. Except one patient with factor X deficiency, all patients had one bleeding episode. Three patients had a high titer inhibitor against factor VIII. The most important factor was trauma. A history of recent trauma was documented in 15 patients. Intracerebral and subdural hematoma were more frequently seen. The most frequent symptoms were seizure and headache. The diagnosis of hemophilia was established in 5 patients after intracranial hemorrhage who referred to our center with ICH. In 11 patients, hematoma was evacuated. Three hemophilia patients died due to ICH and 5 patients presented late sequelae.

Conclusion: Intracranial hemorrhage is the most serious complication in childhood, especially for hereditary bleeding disorders. Urgent establishing diagnosis and treatment with prompt doses of factors to initially maintain a normal concentration of circulating factor is mandatory. Despite the prompt treatment, death and late sequelae can be seen in this patients group.

Disclosure of Interest: None Declared.

PO303-WED

A prospective Italian survey on intracranial hemorrhage in hemophilia patients: changes over time

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Background: A retrospective study on intracranial hemorrhage (ICH) in Italian hemophiliacs was published previously. The annualized event rate (AER) was 2.50 events per 1000 patients; severity of hemophilia and presence of inhibitor were risk factors for ICH.

Aims: A new Italian prospective survey was conducted so as to verify incidence, mortality, management and risk factors for ICH.

Methods: Data from 2009 to 2014 were collected in a electronic case report form identifying the patients by an anonymous number.

Results: 22 ICH events were reported in 4630 patients with an AER of 0.79 per 1000 patients (95% CI 0.45-2.90). According to age distribution, 7 events occurred in children younger than 4 (AER 8.57 95% CI 3.57-14.6) and 15 in subjects older than 35 (AER 1.03 95% CI 0.64-4.25). Children were all affected by severe hemophilia (in 3 hemophilia was diagnosed for ICH event), 3 children were on demand and one was positive for inhibitor. Head trauma was reported in 71.4% of them. The mortality rate (MR) was 42.8% and 2 children presented with permanent neurological sequelae (PNS). Among the 15 adults with ICH, 5 patients had severe hemophilia, 2 moderate and 10 (66.7%) suffered from a mild disease. As to patients with moderate-severe hemophilia, 2 had inhibitors and all except one were not on prophylaxis at the time of ICH. Among the 10 mild hemophilia patients, they were all on on-demand and had hypertension. 7 patients died (MR 46.7%) and 4 had PNS.

Conclusion: ICH is still the most important complication in hemophilia with high mortality rate and morbidity. In newborns head trauma and severe hemophilia are risk factors for ICH onset. No cases have been reported among adolescents and young adults. Longer life expectancy and consequent co-morbidity typical of the elderly can play a key role in ICH occurrence in mild hemophilia adults. Being different from prior published data, such results may be associated with changes in treatment strategies, particularly with the widespread provision of prophylactic regimens.

Disclosure of Interest: None Declared.

PO304-WED

Combination of biomarkers for assessment of hemophilic arthropathy

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Background: Hemophilic arthropathy (HA) is the major complication in patients with hemophilia (PWH). Some serum or urinary biomarkers for cartilage and bone damage have been reported to have significant correlation with severity and progression of joint degeneration.

Aims: This study were to explore whether the combination of various commercially available biomarkers have good correlation with plain X-ray (Pettersson score) and sonographic score.

Methods: We prospectively enrolled 131 consecutive PWH (A: 124, B: 7) with 83 severe, 23 moderate and 25 mild type from June 2011 to July 2014. Their median age was 32 years old with a ranged of 5 to 66 years old. The assessment of each patient's HA comprised radiological tools with X ray Pettersson score and sonographic score of 6 index joints and biomarkers measurement. Five serum or urinary biomarkers including serum C-terminal cross-linking telopeptide of collagen type I (CTX-I), tartrate-resistant acid phosphatase-5b, osteocalcin, cartilage oligomeric matrix protein (COMP) and urinary C-terminal cross-linking telopeptide of collagen type II.

Results: Both serum CTX-I and osteocalcin had moderate negative correlation with the Pettersson score, respectively ($r = -.459$, $P < 0.0001$; $r = -.698$, $P < 0.0001$). There was moderate correlation between serum COMP level with joint Pettersson score ($r = 0.452$, $P < 0.0001$). There was moderate correlation between age and joint Pettersson score ($r = 0.573$, $P < 0.0001$). There was no correlation between sonographic score and X ray Pettersson score for HA assessment. By multivariate analysis, only age was found to have positive association with joint severity ($P < 0.001$).

Conclusion: Our study demonstrate that some of serum or urinary biomarkers had moderate positive or negative correlation with the severity of HA evaluated by conventional X ray Pettersson score, which may be due to age effect. There was no correlation between Pettersson score and sonographic score for HA evaluation.

Disclosure of Interest: None Declared.

PO305-WED

How haemophilia patients in Germany perceive their actual treatment and what they expect for future treatment with new long-lasting products

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Background: Prophylactic treatment allows patients with haemophilia (PWH) to live a normal life, however the need of frequent weekly injections may impact negatively treatment adherence which might result in increased annual bleeding rate. Therefore there is a need for half-live extended products which will be launched in the next years.

Aims: The German patient organisation DHG is interested to better understand PWH's expectations and concerns towards these new products in order to adapt their support for the haemophilia community.

Methods: The study consists of two phases: 1) conduct of separate focus groups with adult haemophilia patients and parents of haemo-

philic children with respect to the new long-lasting products; 2) systematic postal survey among DHG members with haemophilia A or B ($n = 1,500$).

Results: Five focus groups were conducted, three with 23 adult haemophilia patients (mean age 40.96 ± 17.3 years, range 19-71) and two with 9 parents of haemophilic children ranging from 4-19 years (55.6% were mothers; mean age 45.11 ± 5.1 years, range 39-52). The major part had haemophilia A (78.3% of adults, 88.9% of children) and were severely affected (86.4% of adults, 77.8% of children). Patients would like to have a prolonged half-live, a better stability and an easier handling of their actual product. Parents would like to have a better transparency concerning the production of the current product and to improve its manageability. Patients and parents have almost no information about the new up-coming products, wish more information about dosing and safety and are willing to change product only if the benefits are significantly better than with the actual product and the prolongation of the half-live is double as high as the actual product.

Conclusion: This survey, based on representative focus groups from all parts of Germany, will give conclusive answers from a large cohort of haemophilia patients on the real needs of PWH with regard to the upcoming long-lasting products.

Disclosure of Interest: S. Von Mackensen Grant/Research Support from: SOBI, H. Struck: None Declared, W. Kalnins: None Declared.

PO306-WED

Challenges in the diagnosis and management of haemophilia

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Background: The diagnosis and management of Haemophilia can be challenging when inhibitors develop, or haemophilia is diagnosed in unusual situations like female haemophilia. Because of its impact on patient's morbidity, inhibitors is the most serious complication of haemophilia A treatment. We studied all the cases who presented with bleeding manifestations to our institution which is a tertiary care referral centre.

Aims: To highlight the challenges in the diagnosis and management of hemophilia, with special emphasis on inhibitors which constitute the most commonly encountered challenges.

Methods: The diagnosis of hemophilia A was confirmed by Factor VIII:C Assay and the presence of inhibitors were quantitated by the Bethesda assay and correlated with clinical details

Results: The unusual cases of haemophilia which posed diagnostic challenges are described. Mild haemophilia (factor levels $>5\% < 30\%$) can be challenging and it is recommended to run the assay with a very sensitive APTT reagent to detect the mild prolongation of APTT and mild factor deficiency. Two cases of haemophilia A in females were diagnosed, one of which was a patient with 46 XO chromosome pattern and the other with extreme lyonisation of the X chromosome. Haemophilia carrier was diagnosed in one case based on coagulation tests and clinical presentation and family history. Inhibitors to factor VIII:C were diagnosed by Bethesda assay in 23% of the cases. 4 cases of inhibitors were detected in children. Acquired haemophilia A was diagnosed in 6 cases by performing quantitation of factor VIII inhibitors by Bethesda assay. The spectrum of cases will be discussed in detail in the paper.

Conclusion: Recognition of unusual clinical situations in haemophilia and timely detection of inhibitors to factor VIII:C will play a crucial role in management. Acquired haemophilia A, although rare is associated with life threatening haemorrhage and needs prompt diagnosis and appropriate management to reduce morbidity.

Disclosure of Interest: None Declared.

PO307-WED

Factor XI deficiency and bleeding risk on anticoagulationChapin J¹, Klute K¹, Christos P² and DeSancho M¹¹Hematology-Oncology; ²Weill Cornell Medical College, New York, USA

Background: Factor XI (FIX) deficiency is a rare bleeding disorder characterized by low levels of FXI and variable bleeding patterns. Disease severity is classified as severe FXI:C $\leq$ 15% or partial >15-64%. Anticoagulation (AC) and antiplatelet (AP) agents are used to prevent thrombosis and stroke, although there is little data to guide their use in FXI deficiency.

Aims: To determine bleeding and thrombotic events in a single center population of FXI deficiency patients on AC and AP agents.

Methods: A retrospective chart review of adult patients with FXI deficiency presenting to an urban tertiary referral center from 2010-2014 was performed. Data extracted included age, comorbidities, bleeding, FXI activity, AC/AP use, and thrombosis history. Bleeding related to AC/AP was based on chart review.

Results: We identified 38 patients with FXI deficiency in our center, with a median age of 37 years (range 22-88). The median FXI activity was 37% (range 1-59%). We identified 13 patients with severe FXI deficiency and 25 with partial deficiency. 23 had bleeding symptoms at baseline (12 partial and 11 severe; $P = 0.03$). We identified 15 FXI deficiency patients using AC or AP agents including aspirin ($n = 7$), warfarin ($n = 1$), aspirin & clopidogrel ($n = 2$), aspirin & warfarin ($n = 1$), aspirin, clopidogrel & warfarin ($n = 1$), low molecular weight heparin (LMWH; $n = 2$), and LMWH & aspirin ($n = 1$). We found 5 patients with partial deficiency had bleeding related to AC/AP exposure, including patients on LMWH ($n = 2$), warfarin ($n = 1$), aspirin ($n = 1$), and aspirin & LMWH ($n = 1$). There were no bleeding events in the 5 patients on AC/AP therapy with severe deficiency.

Conclusion: In this small retrospective single center study, we found 5 bleeding events related to AC/AP in partial FXI deficiency. We did not find increased bleeding rates on AC/AP in severe FXI deficiency. All 3 FXI deficiency patients on LMWH experienced bleeding. Larger longitudinal cohort studies are needed to determine AC/AP safety in this population.

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PO308-WED

The effect of advancing age on the response to DDAVP in re-exposed nonsevere hemophilia A patientsLoomans J¹, Van Velzen AS¹, Peters M¹, Königs C², Mäkipernä A³, Platokouki H⁴, Mauser-Bunschoten EP⁵, Male C⁶, van Gorkom BAL⁷, Haya S⁸, Cnossen MH⁹, Van der Bom JG¹⁰, Fijnvandraat K¹ and on behalf of RISE consortium

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Background: Large inter-individual variation in DDAVP response is observed in nonsevere hemophilia A patients. Age partially explains the observed variation, since factor VIII levels usually increase with age. Hence, older patients may show a slightly better response compared to younger patients. However, limited data is available on the effect of advancing age within re-exposed patients, as patients are mostly tested once in their lifetime.

Aims: To evaluate age and the response to DDAVP among re-exposed nonsevere hemophilia A patients.

Methods: The RISE study is an international cohort study including data of 1210 nonsevere hemophilia A patients with known DDAVP test results from 23 Hemophilia Treatment Centers in Europe, Canada and Australia. Main outcome is the absolute increase of factor VIII activity (FVIII:C) upon DDAVP stimulation from baseline to peak FVIII:C level. Linear mixed model analysis, using a random intercept and slope was used to study the association between age and response to DDAVP within patients re-exposed to DDAVP with a time-interval of ≥ 2 years. Subjects served as their own controls.

Results: Data on two DDAVP test results ≥ 2 years apart was available for 112 patients. Median age at time of first DDAVP exposure was 15 years (IQR 5-36) and 24 years (IQR 13-58) at the second exposure. Median FVIII:C before and after DDAVP at first exposure was respectively 14 (IQR 7-25) and 44 (IQR 23-65) IU/dL and at the second exposure it was respectively 15 (IQR 8-24) and 49 (IQR 25-69) IU/dL. We observed a trend towards an association between age and response to DDAVP ($P = 0.057$), adjusted for baseline FVIII:C. Lack of significance may be caused by limited sample size.

Conclusion: Increasing age showed a trend for association with the absolute increase in factor VIII activity following DDAVP administration within this group of nonsevere hemophilia A patients that received multiple exposures to DDAVP over time.

Disclosure of Interest: None Declared.

PO309-WED

Follow up of factor VIII inhibitor kinetics in hemophilia patients

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Background: The kinetic feature of coagulation factor VIII inhibitor is classified into type I and type II. Ling et al. demonstrated that by diluting patient's plasma to measure residual FVIII activities, inhibitor kinetic could be differentiated efficiently.

Aims: We examined the type 1 and 2 kinetic characteristics of FVIII inhibitor from residual FVIII activities as the underlying measurements for FVIII inhibitor assay and followed up their changes.

Methods: We reviewed the primary data of the past FVIII inhibitor assays and plotted log scaled residual FVIII activities vs. plasma dilutions to classify their curve fits into linear and nonlinear complex patterns.

Results: Fifty eight past inhibitor assays of 13 patients with hemophilia A performed from 2006 to 2014 were reviewed for the appearances of residual FVIII activity curve. Of 13 patients, 11 were genetically caused by F8 mutation and 2 were acquired hemophilia. Inhibitor titers were followed up in 9 patients for 41 months in average. Eight out of 58 inhibitors tested could be classified as quasi-type I. For 6 inhibitors tested, the curves were clearly linear but the residual activities were well over 2% at the highest titers. Nine inhibitors demonstrated type II like kinetic curves. Two of them were from an acquired hemophilia patient. Another acquired hemophilia patient showed seemingly non-linear curve of decline but no plateau part was recognizable and inhibitor titer were all lower than 5 BU mL⁻¹. Inhibitors not classified were from around 1 to 9.4 BU mL⁻¹ and assumed equivocal pattern in curve appearance. Interestingly, in two patients we could observe the transition from quasi-type II kinetic to near type I linear decline during follow up. And in one, the curve from the last assay showed a plateau in the mid portion of the curve assuming a chair-like shape.

Conclusion: Plotting residual FVIII activity curve in FVIII inhibitor assay can often provide information about the kinetic nature of the inhibitor.

Disclosure of Interest: None Declared.

PO310-WED

The potential correlation between patient-reported symptoms and the need for additional hemostatic medication; a post hoc exploratory analysis based on RFVIIA data from the ADEPTTM2 trial

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Background: Hemophilia treatment guidelines advocate early on-demand treatment of bleeds to optimize outcomes. As such, patients with hemophilia are encouraged to treat their bleeds at home as soon as they think a bleed has occurred (as indicated by their symptoms). In the adeptTM 2 trial, information on self-reported bleeding symptoms was collected together with detailed bleed-related data. Treatment with 1–3 doses of rFVIIa 90 µg kg⁻¹ was efficacious in 93% of all bleeds and in 92% of all joint bleeds 12 h after the first dose. However, when evaluated at 12–24 and 24–48 h, additional hemostatic medication

(AHM) was used in relation to a higher proportion of joint bleeds vs. all bleeds.

Aims: To investigate the relationship between patient-reported symptoms and the use of AHM. We also analysed how frequently symptoms were reported for joint bleeds in order to gain a better understanding of how to collect patient-reported symptoms in future trials of patients with hemophilia A or B with inhibitors.

Methods: In total 177 joint bleeds (out of 227 total bleeds), and patient-reported symptoms pain, Visual Analog Scale for pain, swelling, mobility, tingling, and warmth were investigated. Statistical analyses were performed to describe the relationship between symptoms and the use of AHM; information was gathered on how many patients reported a symptom and how the symptom changed over time (up to 9 h post first dose) in patients who took AHM vs. those who did not. A logistic regression model was used to estimate the probability that a bleed would be treated with AHM, based on the explanatory variables. The correlation between additional treatment within subjects was modelled using a subject-related random intercept in the logistic regression, which addresses the over-dispersion caused by between-patient differences in treatment patterns.

Results: These results will be submitted as soon as available, and presented at the meeting.

Conclusion: Conclusions will be submitted and presented at the meeting.

Disclosure of Interest: S. Lentz Consultant for: Consultant for Novo Nordisk, S. Rangarajan Consultant for: Consultant for Novo Nordisk, F. Karim Consultant for: Consultant for Novo Nordisk, P. Andersen Employee of: Novo Nordisk A/S, P. Arkhammar Employee of: Novo Nordisk A/S, G. Rosu Employee of: Novo Nordisk A/S, J. Mahlangu Consultant for: Consultant for Novo Nordisk.

Heparin-induced thrombocytopenia (HIT) II

PO311-WED

Efficacy assessment of fondaparinux treatment of patients with heparin induced thrombocytopenia (HIT)

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Background: HIT is a clinical syndrome which main symptom is an acute platelets count fall. Clinical observations are confirmed by detection of antibodies of the IgG class dependent on Heparin by means of a sensitive and specific method

Aims: to assess the efficacy and safety of fondaparinux treatment in heparin-induced thrombocytopenia. Additionally, the efficacy of dosing 1 × 7.5 mg was tested

Methods: Initially, a group of patients with confirmed Deep Vein Thrombosis (DVT) or with newly diagnosed pulmonary embolism (PE) was selected, all of whom were treated by Low Molecular Weight Heparin (LMWH) or unfractionated heparin (UFH) in our Unit, in 2006-2014. From the group selected, all patients were further distinguished who fulfilled clinical criteria of HIT (the 4T score) and with confirmed presence of antibodies against platelet factor 4 (PF4) heparin complexes

Results: 15 patients were assured as confirmed HIT cases. There were 6 women and 9 men. 10 patients were treated for PE and 5 patients for DVT. 80% were given LMWH and 20% - the infusion of UFH. The maximum platelets concentration fall in the third day of the therapy amounted to 220.000 (from 300.000 to 80.000), the average platelets concentration fall – 110 thousand and the minimal platelets concentra-

tion fall – 30 thousand. After the fondaparinux treatment (1×7.5 mg) was implemented, there was observed an increase of platelets concentration in all cases, while the increase of platelets up to the initial value was noticed among 5 patients (45%). Despite the treatment implemented there was noted a thrombosis recrudescence among 4 patients, out of which 2 died for acute pulmonary embolism.

Conclusion: Fondaparinux treatment occurred an effective procedure as all patients experienced the platelets concentration increase however, among only 5 patients (45%) the concentration gained initial value. Treatment was also safe since it did not lead to adverse reactions of bleeding

Disclosure of Interest: None Declared.

PO312-WED

Safety and efficacy of starting warfarin after two consecutive platelet rises in patients with heparin-induced thrombocytopenia

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Background: Current guidelines for heparin-induced thrombocytopenia (HIT) treatment recommend warfarin initiation when platelet levels recover to $150 \times 10^9/L$ or more. At the University of California Davis Medical Center (UCDMC), warfarin is generally initiated after two consecutive upward trending platelet levels, regardless of absolute platelet count. The potential benefit of starting warfarin therapy earlier is shorter hospital length of stay (HLOS) and reduced health care costs.

Aims: To determine the safety and efficacy of initiating warfarin when platelets start trending upward instead of at a specific level for patients with HIT.

Methods: Patients hospitalized between January 2008 and November 2014 with a diagnosis of HIT were assessed. HIT was defined as a positive serotonin-release assay, heparin ELISA test with optical density (OD) greater than or equal to 1.4, or an OD of 0.4 to 1.3 and 4-Tscore of four or more. Patients less than 18 years of age, pregnant or breast feeding, cognitively impaired, prisoners, taking warfarin prior to HIT diagnosis, or with contraindication to warfarin were excluded. The primary outcome was incidence of new thrombosis within 30 days of starting warfarin. Institutional Review Board approval was obtained.

Results: Fifty-eight of 598 patients met pre-defined criteria. Warfarin was started prior to platelets reaching $150 \times 10^9 L^{-1}$ in 30 patients. One patient developed new thrombosis after starting warfarin. Three patients passed away due to causes unrelated to HIT. The average HLOS for patients who started warfarin at platelets less than $150 \times 10^9/L$ compared to current guidelines was 22.2 ± 12.7 and 38.8 ± 19.1 days respectively ($P = 0.0002$).

Conclusion: The absolute platelet level at which warfarin is initiated does not affect the rate of thrombosis or mortality but can shorten overall HLOS. Therefore, it may be more important to observe an upward trend in platelets rather than striving to achieve an absolute platelet level before initiating warfarin in patients with HIT.

Disclosure of Interest: None Declared.

PO313-WED

Rapid functional heparin induced thrombocytopenia (HIT) assay using multiplate: comparison with heparin induced platelet activation (HIPA) assay and ACL AcuStar antigen assay

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Background: Patients treated with heparin can develop the severe life threatening complication HIT. HIT is caused by platelet-activating antibodies (Ab) against platelet factor 4/heparin (PF4/hep) complexes. Rapid detection of PF4/hep Ab using antigen assays is helpful in patient management, however unlike activation assays; antigen assays suffer lower diagnostic specificity and are subject to false positive findings. Classical functional assays are time consuming and technically challenging limiting their use to specialised laboratories.

Aims: To evaluate a rapid semi-automated functional assay Heparin Induced Multiple Electrode Aggregometry (HIMEA) (Multiplate) for confirmation of HIT.

Methods: A total of 230 patients with suspected HIT were assayed using antigen assay HemosIL® AcuStar HIT-IgG_(PF4-H) (Instrumentation Laboratory, Bedford, MA, USA), and 2 functional assays HIMEA (Multiplate – Elalamy et al. 2009) and the 'gold standard' HIPA assay (Greinacher et al. 1991).

Results: HIPA: 30 of 230 positive (including 8 non classical positives).

AcuStar HIT-IgG: Using the recommended cut off (of 1U/mL), positive results obtained in 45 of 230 samples, including all 30 which were reactive by HIPA. Sensitivity and negative predictive value (NPV) 100%, specificity 92.5% and positive predictive value (PPV) 66.7%.

HIMEA: Positive results obtained in 31 of 230 (including 5 non classical positives) including all 30 which were reactive by HIPA. Sensitivity and NPV 100%, specificity 99.5% and PPV 96.8%.

Conclusion: HIMEA functional assay performs favourably in comparison to the antigen assay, high-lighting the need to confirm positive antigen assay results. However, the false positive rate with ACL AcuStar™ was lower than those reported for a number of other antigen assays. The rapid and semi automated nature of the HIMEA provides a realistic alternative to other highly sensitive and specific functional assays as a means of a timely confirmation test, however it is still hampered by the requirement for reactive donor platelets.

Disclosure of Interest: None Declared.

PO314-WED

Immunological assays for diagnosis of heparin-induced thrombocytopenia: a systematic review and meta-analysis

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Background: Immunoassays are essential for diagnosis of heparin-induced thrombocytopenia (HIT) in clinical practice. However, the diagnostic value with regard to different assays and test variations is uncertain.

Aims: To assess the diagnostic accuracy of immunoassays for diagnosis of HIT for different assays and test variations.

Methods: Systematic review: Papers were searched in electronic databases (Medline, Embase, Cochrane library) and reference lists of included studies. The QUADAS-2 tool was used to assess methodological quality of included studies. Data to fill the two-by-two tables were

obtained. Diagnostic meta-analyses using the unified method were performed.

Results: Out of 2716 articles, 256 were assessed in full-text for eligibility. After exclusion with reasons, 49 articles (127 test evaluations) were included in systematic review. A low risk of bias according to QUADAS was observed in 4 studies only (3.1%). Data of 87 test evaluations comprising 22,715 patients were included in meta-analysis. Pooled sensitivity ranged from 91.2% to 98.9%, and specificity from 85.4% to 97.8% for polyspecific ELISA, IgG specific ELISA, particle gel immunoassay, lateralflow immunoassay, polyspecific chemiluminescent immunoassay, and IgG specific chemiluminescent immunoassay. Positive likelihood ratio ranged between 6.7 and 41.4 and negative likelihood ratio between 0.01 and 0.09. Both increased with higher thresholds. However, sensitivity was limited with regard to high thresholds and high dose heparin confirmation step.

Conclusion: Methodological quality of studies investigating diagnostic accuracy of antibody tests for HIT is limited. However, diagnostic accuracy seems adequate for clinical practice in most tests investigated. Physicians should be aware of the pre-test probability (prevalence), which determines the clinical value of diagnostic tests to a large extent.

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PO315-WED

Impact of overdiagnosis of HIT in a large community hospital

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Background: Heparin Induced Thrombocytopenia (HIT) is a serious complication of heparin requiring prompt recognition/treatment. Diagnosis is supported with validated tools including 4T score, detection of heparin-PF4 antibodies (ELISA) and serotonin release assay (SRA). The high frequency of thrombocytopenia and HPF4 antibodies after cardiac surgery may lead to HIT overdiagnosis, especially when a pan-specific ELISA is employed. In our institution, bivalirudin (BV) is the preferred HIT therapy, and is the highest budget-consuming drug on Formulary.

Aims: To examine BV use in our institution, especially for HIT treatment, in order to recognize appropriate use and patient safety issues, and to identify areas for education or improvements in the use of BV.

Methods: We reviewed 82 records from patients receiving at least one dose of BV between 7/1/14 and 9/30/14 (pediatric patients, patients undergoing chemotherapy, and cath lab use were excluded).

Results: Forty two (51.2%) patients received BV for suspected HIT, while 40 were treated for non-HIT indications (11 had HIT history, while 29 either had no history or previous, unconfirmed suspicion of HIT). Patients treated for HIT had 79 ELISAs performed (0 tests n = 2; 1 test n = 20; 2 tests n = 9; 3 tests n = 6; 4 tests n = 3; 5 tests n = 1; 6 tests n = 1). Of these, the ELISA OD was ≤ 0.4 (n = 12); 0.41-1.0 (n = 22); >1.01 (n = 5). BV was used for 613 patient days in 82 patients. Median treatment duration was 5 days (range 1-50) and 27 patients used at least one unit of blood products.

Conclusion: We conclude that there are areas for improvement in BV use. These include overdiagnosis of HIT, inappropriate baseline APT-Ts leading to higher therapeutic ranges, and lack of discontinuation following a negative assay. These results point to the need for a collaborative HIT management protocol including a pharmacy consult, 4T score, appropriate interpretation of an IgG-specific ELISA and reflex SRA testing, to reduce HIT overdiagnosis, unnecessary bleeding risks and excessive costs.

Disclosure of Interest: None Declared.

PO316-WED

New platelet functional method to identify pathogenic antibodies in HIT patients

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Background: Heparin-induced thrombocytopenia (HIT) is a thrombotic complication of heparin therapy. HIT is an autoimmune disorder characterized by reduction in platelets count. Clinical estimation and laboratory tests are the more appropriate approach to HIT diagnosis. Functional assay is necessary for a correct HIT detection. Among such tests 14C-serotonin release is the gold standard (SRA). Luminographic detection of platelet ATP release was also suggested, however it requires a specific apparatus.

Aims: We describe an alternative functional method in suspected HIT serum measuring platelet ATP release using luciferin/luciferase assay that have a stable luminescent signal, and evaluate it with multilabel plate reader.

Methods: Our studied population consists of patients previously tested with PF4-elisa test, positive serum (N = 19) and negative serum (N = 20). Patients' serum was incubated in platelet free plasma (PFP) in the presence of low heparin dose (LHD 0.1U/ml) and high heparin dose (HHD 100U/ml) for 20 min at RT and ATP released was measured with luciferin/luciferase assay (ATPlite). Dates are reported as ratio between LHD/HHD of Luminescence AU. Control positive secretion was detected using U46619 plus Epinephrine (2 + 20 μ M) as agonist. The same serum samples were tested for SRA.

Results: The range ratio of negative samples is from 0.6 to 1.8. The range ratio of positive samples is from 2.1 to 4.7 and for positive samples without thrombocytopenia (N = 5) the range ratio is from 1.4 to 2.4. Parallel behaviour was obtained with SRA test. The range ratio of negative samples is from 0.7 to 1.0. The range ratio of positive sample is from 1.1 to 4.5 and for samples without thrombocytopenia the range ratio is from 0.5 to 1.2.

Conclusion: ATP release using a stable luciferin/luciferase is a valid functional method to detect HIT pathogenic antibodies. It does not require any radioactive products or specific apparatus. We propose that it will become a gold standard.

Disclosure of Interest: None Declared.

PO317-WED

Low molecular weight heparin treatment for superficial vein thrombosis and the risk of heparin-induced thrombocytopenia

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Background: The risk of heparin induced thrombocytopenia (HIT) associated with low molecular weight heparin (LMWH) treatment of superficial vein thrombosis (SVT) is not known.

Aims: To assess the risk of HIT in outpatients with SVT treated with LMWH.

Methods: In a retrospective single centre study we included all 375 symptomatic outpatients (age 66.5 ± 15.8 years, male 42.4%) in whom a real-time B-mode and color Doppler ultrasonography examination revealed a SVT. We excluded patients with renal insufficiency (GFR < 30 mL min⁻¹), or patients receiving vitamin K antagonists or fondaparinux. Patients received full dose LMWH for 1 week followed by half therapeutic dose LMWH for 3 weeks. Platelet count was performed on the day of diagnosis (D0) and 7 (D7), and 14 (D14) days afterward. The primary end-point was the rate of HIT, the secondary end-point the rate of major bleeding.

Results: During LMWH treatment no major bleeding was observed. Platelet count was $259 \pm 118 \times 10^9/L$ at D0, $237 \pm 103 \times 10^9/L$ at D7, and $272 \pm 189 \times 10^9/L$ at D14. No patient had a fall in platelet count >50% and no HIT case was observed (HR 0.0% CI95%: 0-0.97%).

Conclusion: The 4-week LMWH treatment of SVT is associated with a risk of HIT <1%. As for 'medical/obstetrical patients' receiving LMWH, routine platelet count monitoring may be not necessary.

Disclosure of Interest: None Declared.

PO318-WED

Audit of test results following the introduction of a testing algorithm for heparin induced thrombocytopenia (HIT) in a referral laboratory

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Background: Antibodies to platelet factor 4/heparin complexes can cause HIT increasing the risk of venous/arterial thrombosis. The pre-test probability for HIT (4T score) is calculated as low (1-3), intermediate (4-5) or high (6-8). Laboratory testing comprises immunological and functional tests.

Aims: We reviewed the utility of a three test panel and algorithm for HIT testing based on recent British Committee for Standards in Haematology guidelines including the requirement for the 4T score with each request.

Methods: HIT was excluded in patients where the score was ≤ 3 . All other requests were tested by a rapid test (ID-PaGIA Heparin/PF4 Antibody Test, Biorad) and by ELISA for the detection of PF4 antibodies (Lifecodes). Positive ELISA samples with demonstrable heparin dependence were further tested by washed platelet aggregation assay (HIPA). A positive result in all three tests was considered positive for HIT.

Results: 189 requests with a 4T score were reviewed, 14/189 were ≤ 3 and were omitted from testing. *Intermediate 4T score (n = 109)*. 5 samples were positive with all three tests. 35 samples were positive with the rapid test, 12/35 were positive by ELISA; 4 with intermediate Optical Density (OD) of 0.400–1.00 and 8 with high OD of > 1.00 . No samples with intermediate OD and 5 with high OD were positive by the HIPA test and positive for HIT. *High 4T score (n = 66)*. 6 samples were positive with all three tests. 17 samples were positive with the rapid test. 12/17 were positive by ELISA; 3 with intermediate OD and 9 with high OD. No samples with intermediate OD and 6 with high OD were positive by the HIPA test and positive for HIT.

Conclusion: The likelihood of detecting platelet activating antibodies by the HIPA test increases when the measured OD by ELISA is >1.00 . The HIPA test is useful for the detection of clinically significant antibodies. A testing algorithm where HIT can be excluded based on the ELISA method allows a rapid turnaround in HIT testing and streamlined testing.

Disclosure of Interest: None Declared.

Immune thrombocytopenia (ITP) III

PO319-WED

Clinical significance of antinuclear antibody test and risk factor predictors of childhood chronic immune thrombocytopenia

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Background: Immune thrombocytopenia (ITP) is an autoimmune disease characterized by persistence thrombocytopenia due to antibody binding to platelet antigen causing platelet destruction by reticuloendothelial system. Currently, there is lack of the relevant risk factors for predictor of chronic ITP (CITP).

Aims: To determine the significance of the antinuclear antibody (ANA) test and risk factor predictors in childhood CITP.

Methods: We reviewed the medical records of patients who were diagnosed with ITP at age of 1–15 years at Siriraj Hospital between January 2007 and December 2012.

Results: One hundred and three patients were enrolled (46% were newly diagnosed and persistent ITP and 54% were CITP). Seventy percent of patients had ANA testing. Twenty two percent of this group had positive test for ANA (ANA+). The patients with ANA+ and absolute lymphocyte count (ALC) $\leq 1500/\mu L$ were likely to develop CITP but no statistical significance ($P = 0.365$, OR 2.05, 95%CI 0.51–8.11 and $P = 0.176$, OR 3.21, 95% CI. 0.63–16.28, respectively). The other risk factors to develop CITP were patients' age over 10 years at diagnosis ($P = 0.039$, OR 3.07, 95%CI 1.02–9.23) and the patients with severe bleeding score: grade 3–4 ($P = 0.049$ OR 2.39, 95%CI 0.99–2.26). We analyzed the factors that predict acute ITP course by multiple logistic regression and found that febrile illness preceded the onset of ITP ($P = 0.006$, OR 3.61, 95%CI 1.45–9.09) and the first treatment with intravenous immunoglobulin (IVIG) ($P = 0.025$, OR 4.5 95%CI 1.20–17.24) were significant factors.

Conclusion: Childhood ITP with ANA+ and ALC $\leq 1500/\mu L$ trend to have chronic ITP course. The age over 10 years and patients presented with severe bleeding score were the significant risk factors to develop chronic ITP. The patients with febrile illness preceded the onset of ITP and received the first treatment with IVIG found to have significantly acute ITP course. Further study with more number of patients and prospective way may answer this question.

Disclosure of Interest: None Declared.

PO320-WED

Clinical significance of IPF% or RP% measurement in distinguishing primary immune thrombocytopenia from aplastic thrombocytopenic disorders

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Background: The diagnosis of primary immune thrombocytopenia (ITP) is based on differential diagnosis. Although the measurement of percentages of reticulated platelets (RP%) by flow cytometry is useful as a supportive diagnostic test, this method is nonetheless a time-consuming, laboratory-based assay.

Aims: To identify alternative assays that are useful in daily practice, we compared three methods in parallel, IPF% measured by XE-2100

[IPF% (XE), Sysmex corp.], IPF% measured by new XN-1000 [IPF% (XN)], and RP%.

Methods: We examined 47 patients with primary ITP, 28 patients with aplastic thrombocytopenia [18 aplastic anemia and 10 chemotherapy-induced thrombocytopenia] and 80 healthy controls. In a selected experiment, we examined 16 patients with paroxysmal nocturnal hemoglobinuria (PNH) to examine the effect of hemolysis. We obtained informed consent from all subjects, in accordance with the Declaration of Helsinki. Automated hematology analyzers, XE-2100 and XN-1000, were supplied by Sysmex corp. during this study. This study was approved by Osaka University Institutional Review Board.

Results: As compared with IPF% (XE), IPF% (XN) showed better within-run reproducibility. The sensitivity and specificity for the diagnosis of ITP were 83.0% and 75.0% for IPF% (XE), 85.1% and 89.3% for IPF% (XN), and 93.6% and 89.3% for RP%, respectively. Examination of PNH patients revealed that hemolysis and/or red blood cell fragments interfered with IPF% (XE) values, but not with IPF% (XN) values.

Conclusion: Our results suggest that IPF% measured by XN-1000 may be of comparable value with RP% as a supportive diagnostic test for ITP.

Disclosure of Interest: None Declared.

PO321-WED

Intravenous immunoglobulin treatment of children immune thrombocytopenia Meta analysis of efficacy and safety in CHINA

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Background: In China, the incidence of children immune thrombocytopenia is higher, but the lack of effective evidence for the clinical efficacy and safety reference for the drug treatment of ITP.

Aims: Using evidence-based medicine method to evaluate drug efficacy and safety of the treatment of children immune thrombocytopenia in China. To provide the reference for the pediatric clinical application.

Methods: Retrieval of China published intravenous immunoglobulin and glucocorticoid treatment of children immune thrombocytopenia randomized controlled trial (RCT) from January 1, 2000 to December 31, 2012. By the evaluators to evaluate about the quality of the included studies and data extraction. Meta analysis using Stata 12.0 software. According to the statistical standards for merger analysis, to evaluate intravenous immunoglobulin and glucocorticoid treatment efficacy and safety of the children with immune thrombocytopenia, and organic combination of evidence-based medicine and pediatric clinical, provide the best evidence for children with individualized treatment.

Results: A total of 12 original articles, 1215 cases, were involved in the Meta-analysis. Using Stata 12 software all the documents to show the efficiency and effectiveness of Meta-analysis. The result of meta-analysis showed that compared with glucocorticoid, intravenous immunoglobulin could increase the total significant rate [$Z = 2.87$, $OR = 1.302$, 95% CI (1.088~1.562), $P < 0.05$], with significant differences, and overall efficacy rate was [$Z = 1.03$, $OR = 1.093$, 95% CI (0.923~1.294), $P > 0.05$], with no statistically difference.

Conclusion: This data showed that intravenous immunoglobulin has high efficiency and security to treat ITP, but this study is needed to carry out more high quality and reliability of large sample research for confirmation. So there is no suitable for their own children evidence-based practice guidelines for ITP in China

Disclosure of Interest: None Declared.

PO322-WED

Variations of mean platelet volume of immune thrombocytopenia

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Background: Thrombocytopenia in immune thrombocytopenia (ITP) is due mainly to platelet destruction, and mean platelet volume (MPV) of ITP patients is larger than that of patients with thrombocytopenia caused by decreased platelet production (e.g., aplastic anemia, post-chemotherapy). Therefore, MPV is a useful marker for the differential diagnosis of ITP. Changes in MPV during the course of ITP, however, are not fully elucidated.

Aims: To examine the MPV variations in ITP patients and analyze the correlation between MPV and platelet count in ITP compared to that of thrombocytopenia caused by decreased platelet production.

Methods: 30 ITP patients and 18 patients with thrombocytopenia caused by chemotherapy, who visited our hospital between April and November 2014, were included. MPV and platelet count were recorded from the medical chart and retrospectively analyzed. The ethics committee of the institutional review board approved the study.

Results: Median platelet count at diagnosis of ITP patients was 45,000/cmm (range 2,000-90,000). MPV (mean $\pm$ SD) at diagnosis of ITP patients was significantly larger than that of post-chemotherapy patients (11.6 ± 1.4 vs 9.7 ± 1.3 , $P < 0.001$). Based on multiple regression analysis, MPV and platelet count were strongly negatively correlated in ITP patients with a coefficient of -0.776, whereas MPV and platelet count were not correlated in post-chemotherapy patients. Platelet levels increased to 100,000/cmm in 18 ITP patients (6 with *Helicobacter* eradication, 5 with prednisolone, 3 with eltrombopag, 1 with high-dose immunoglobulin, and 3 after delivery), and MPV after platelet recovery was significantly decreased compared to that at diagnosis (12.1 ± 1.0 to 10.3 ± 1.0 , $P < 0.001$).

Conclusion: MPV is negatively correlated with platelet count in ITP, and changes might be related to treatment response.

Disclosure of Interest: None Declared.

PO323-WED

The polymorphisms of tumor necrosis factor-induced protein 3 gene may contribute to the susceptibility of chronic primary immune thrombocytopenia in Chinese population

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Background: Primary immune thrombocytopenia (ITP) is an acquired autoimmune disease characterized by decrease of the platelet count and increased risk of mucocutaneous bleeding. Multiple factors have been demonstrated in ITP pathogenesis, including the genetic variants. Tumor necrosis factor-induced protein 3 (TNFAIP3) gene encodes the ubiquitin-modifying enzyme A20 which limits inflammation by terminating NF- κ B activation through several signaling pathways including TNF and Toll-like receptors and regulates immunostimulatory effects of dendritic cells and attenuates antigen presentation. Single nucleotide polymorphisms (SNPs) of TNFAIP3 have been associated with susceptibilities to several autoimmune diseases.

Aims: The aim we conducted this research is to investigate the role of SNPs and haplotypes (rs2230926, rs5029939) of TNFAIP3 in the Chinese patients with chronic primary ITP.

Methods: We investigated the distributions of TNFAIP3 (rs2230926 and rs5029939) polymorphisms in 222 patients with chronic ITP and 153 controls by polymerase chain reaction-restriction fragment length polymorphism and direct sequencing.

Results: We observed significant difference in the allelic and genotypic distributions of rs2230926 and rs5029939 between the ITP and control group ($P < 0.05$). Stratified analysis by gender revealed an association of rs2230926 polymorphism with chronic ITP in male groups. However, none of the two polymorphisms contributed to the onset age of chronic ITP.

Conclusion: In conclusion, our study demonstrated that TNFAIP3 (rs2230926, rs5029939) polymorphisms influenced the susceptibility of chronic ITP in Chinese population, indicating that this gene may be added to the list of genes associated with chronic ITP. Nevertheless, the detailed mechanism has not been figured out. We have been conducting subsequent trials to detect the expression of TNFAIP3 in the DCs and the related signal pathways to elaborate the possible mechanism.

Disclosure of Interest: None Declared.

PO324-WED

Anti-platelet antibodies present in breast milk of an immune thrombocytopenic mother increase an apoptotic marker in normal platelets

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Background: Immune thrombocytopenia (ITP) in pregnant women can cause neonatal thrombocytopenia by transport of anti platelet antibodies across the placenta. Usually, infant's platelet count normalizes within two months. We recently described persistent neonatal thrombocytopenia that lasted more than four months and recovered following discontinuation of breastfeeding (Blood 2014, abstract 124:4181). The persistent thrombocytopenia was associated with anti-platelet antibodies of the immunoglobulin (Ig) A type present in the breast-milk. The antibodies acted against the platelet integrin α Ib β 3 expressed in BHK cells. The mechanism by which the infant's platelet count decreased was not defined.

Aims: To explore whether anti-platelet antibodies found in milk of an ITP patient can induce platelet apoptosis.

Methods: Total Ig was extracted from milk of an ITP woman that exhibited a high titer of anti-platelet antibodies and from five healthy women. The Ig extracts were incubated with platelets of healthy donors. The apoptotic marker phosphatidylserine was measured by annexin V binding using flow cytometry. The platelet activation marker CD62P was also measured.

Results: An excessive level of annexin V binding was observed in response to milk-Ig of the ITP patient compared to milk-Ig of healthy women. This elevation was not accompanied by increased *P*-selectin (CD62P) surface exposure.

Conclusion: Anti-platelet antibodies found in breast-milk of an ITP patient cause an increase of an apoptotic marker but no increase of the platelet activation marker. This result provides a possible mechanism for newborns' platelet destruction and the persistence of thrombocytopenia in breast-fed infants of ITP women.

Disclosure of Interest: None Declared.

PO325-WED

Clinical observation of 10 cases of newly diagnosed itp patients receiving low-dose rituximab combined with high-dose dexamethasone

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Background: Immune thrombocytopenia (ITP) is an autoimmune hemorrhagic disease. It is characterized by decreased platelet count

(<100 × 10⁹/L) caused by immune-mediated platelet destruction. The estimated incidence of ITP is 1.9 to 6.4 per 100,000 per year in children and 3.3 per 100,000 per year in adults. More than half of the new diagnosed ITP patients will develop into chronic ITP.

Aims: To observe the efficiency of low-dose rituximab combined with high-dose dexamethasone for newly diagnosed ITP.

Methods: 10 cases of newly diagnosed ITP patients (1 male and 9 females, median age 26 years, range 13–62 years) were enrolled in this study. All members have signed informed consent, and was approved by the hospital medical ethics committee. All Patients received dexamethasone 40 mg day⁻¹ d1–4, followed by rituximab 100 mg on days 7, 14, 21, 28. Patients were allowed supplemental dexamethasone for up to 3 cycles. Complete response (CR) was defined as PLT ≥ 100 × 10⁹/L, or no bleeding symptoms; response (R) was defined as PLT ≥ 30 × 10⁹ L⁻¹, no bleeding symptoms; No response (NR) was defined as PLT < 30 × 10⁹ L⁻¹, or have bleeding symptoms. The total effective rate (OR) is the sum of CR and R. Observed short-term response rates, sustained response rates efficacy and safety.

Results: Platelet counts of all cases at day 14 remarkably increased, with the median platelet count from 17 (3–32) × 10⁹ L⁻¹ to 105 (25–536) × 10⁹ L⁻¹, overall response was 90% (9/10). And platelet counts were 122 (56–412) × 10⁹/L at day 28, OR was 100% (10/10). The median time to R was 7.5 days, range 3–23 days. The median time to follow-up time is 192 (74–514) days, sustained response rate was 60% (6/10). In follow-up time, 4 cases relapsed after they relieved, the median time to relapse time is 78 (56–22) days.

Conclusion: The results indicate that high-dose dexamethasone combined with low-dose rituximab was an effective treatment for newly diagnosed ITP patients. However, this is a small sample study, we would collect more cases to the further research.

Disclosure of Interest: None Declared.

PO326-WED

Anti-gpIbalpha antibodies induce immune thrombocytopenia in distinct pathways: FC-dependent vs. FC-independent

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Background: Immune thrombocytopenia (ITP) is a common autoimmune bleeding disorder characterized by autoantibody mediated platelet destruction. Platelet α Ib β 3 and GPIb α are the most frequently targeted antigens in ITP. Current theory suggests platelets are engulfed via interaction between the Fc portion of autoantibodies and the Fc γ receptors of macrophages. First line therapy includes intravenous IgG (IVIG) and steroids, but anti-GPIb α mediated ITP are usually less responsive to these therapies although the mechanism is unclear.

Aims: To investigate the mechanisms of anti-GPIb α mediated ITP and its therapy.

Methods: Mouse anti-mouse monoclonal antibodies (mAbs) were generated in GPIb α ^{-/-} mice and in β 3^{-/-} mice. ITP was induced by injection of these mAbs into wild-type (WT) or FcR γ ^{-/-} mice. The effect of these mAbs on platelets was tested by flow cytometry, aggregometry and Western blot.

Results: Novel anti-GPIb α mAbs (NIT A, B, and G) were developed, which induced platelet aggregation and blocked GPIb α -VWF interaction. Through a competitive binding assay we demonstrated that NIT G has a unique binding site as compared to NIT A and B. NIT G, but not NIT A, B and all anti- β 3 mAbs tested, induced ITP in FcR γ ^{-/-} mice, suggesting NIT G-induced ITP is Fc-independent. Consistently, IVIG failed to alleviate ITP in NIT G treated mice although amelioration was observed in NIT A, B and all anti- β 3 mAb mediated ITP.

Furthermore, in the presence of NIT A, B, and $\beta 3$ mAbs, platelets were engulfed by macrophages *in vitro*, while phagocytosis did not occur with NIT G opsonized platelets. Preliminary data showed NIT G, but not NIT A, B or $\beta 3$ mAbs, induced significant α granule release, phosphatidylserine exposure, and P38 MAPK phosphorylation, indicative of platelet activation and apoptosis.

Conclusion: NIT G mimics the effects of Fc-independent autoantibodies in ITP patients. Further investigation into its epitope and the therapy involved may provide insights to treat ITP patients that are resistant to the first line therapies.

Disclosure of Interest: None Declared.

PO327-WED

Comparison of relapse rates of immune thrombocytopenia in patients who received initial treatment with steroids vs. anti-RH(D) with or without steroids

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Background: Steroids and anti-Rh(D) are first-line therapies for immune thrombocytopenia (ITP). Prednisone is the physician-preferred initial treatment of ITP (ASH Friday Satellite Symposium 2008). A randomized study of prednisone vs. anti-Rh(D) with prednisone in newly diagnosed ITP showed increased time to but not prevention of splenectomy in the latter group (George JN AJH 2003). Among 13 patients who required 1 rather than 3 doses of anti-Rh(D) 75 mcg kg⁻¹, 12 averted splenectomy.

Aims: To determine if a single dose of anti-Rh(D) 75 mcg kg⁻¹ with or without steroids as frontline treatment of ITP decreases relapse rates in comparison with steroids alone.

Methods: Institutional review board approval was obtained. The charts of 52 consecutive patients with ITP followed in an adult hematology practice between 07/01/03 and 06/30/13 were reviewed. Inclusion criteria were: 1. Initial date of presentation/treatment known; 2. Platelet count (PLT) < 30,000/ μ L at presentation; 3. Initial treatment with prednisone 1 mg kg⁻¹ daily for 3-4 weeks followed by taper or dexamethasone 40 mg daily for 4 days; 4. Initial treatment with anti-Rh(D) 75 mcg kg⁻¹ with or without prednisone and/or dexamethasone at the aforementioned doses; 5. Date of first relapse (PLT < 30,000 μ L⁻¹) known. Exclusion criterion was pregnancy at initial presentation or at relapse. Chi-square analysis was used to compare 3-year relapse rates.

Results: 6 patients who received steroids (Group 1) and 6 patients who received anti-Rh(D) with or without steroids (Group 2) for initial therapy of ITP were eligible for enrollment. Median age was 55 years (Group 1) and 54 years (Group 2), $P > 0.05$. M:F ratio was 1:2 (Group 1) and 5:1 (Group 2), $P > 0.05$. Rates of relapse at 3 years were: Group 1 100% and Group 2 50%, $P < 0.05$.

Conclusion: A subset of patients who receive a single dose of anti-Rh(D) 75 mcg kg⁻¹ with or without steroids as initial treatment of ITP will have durable remissions. Further utilization of anti-Rh(D) as frontline therapy of ITP should be explored.

Disclosure of Interest: None Declared.

PO328-WED

Pathogenesis of anti-integrin α IIb-mediated fetal and neonatal alloimmune thrombocytopenia: establishment of novel murine models in α IIb deficient and human α IIb transgenic mice

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Background: Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a life threatening bleeding disorder often resulting in intracranial hemorrhage (ICH). Miscarriage has also been reported in FNAIT although the incidence of fetal loss has not been adequately studied. alloantibodies targeting the $\beta 3$ subunit of integrin α IIb $\beta 3$ occurs in 80–90% of reported cases, whereas anti- α IIb mediated FNAIT only accounts for 3–5% of reported cases, although frequencies of polymorphisms in α IIb and $\beta 3$ polypeptides are similar in the human population. It is unknown whether these two types of FNAIT differ and currently no animal model exists to address this question.

Aims: To investigate the pathogenesis and severity of anti- α IIb mediated FNAIT

Methods: To establish a mouse model of anti- α IIb mediated FNAIT, α IIb deficient (α IIb^{-/-}) and human α IIb transgenic female mice were immunized (2-4x) by transfusion with wild-type (WT) mouse platelets and bred with WT male mice.

Results: Miscarriage occurred in the majority of 2x immunized α IIb^{-/-} mothers as detected by ultrasound at E14.5. Vascular abnormalities and poor perfusion in placentae, and low neonatal birth weight were observed in anti- α IIb mediated FNAIT. Severe thrombocytopenia and bleeding (e.g. ICH) were also detected in fetuses and neonates. Antisera from 2-4x immunized α IIb^{-/-} mice enhanced ADP-induced platelet aggregation, whereas anti- $\beta 3$ antisera inhibited this process. Our preliminary data also showed that these antisera inhibited endothelial cell migration and invasion. Preliminary studies with an alloimmune FNAIT model using humanized α IIb transgenic mice showed similar, albeit milder, systemic pathologies.

Conclusion: We established the first anti- α IIb models of FNAIT and the first alloimmune model of FNAIT. Neonates and fetuses exhibited ICH and developmental abnormalities, and most mothers suffered miscarriages. This study demonstrates the severity of α IIb mediated FNAIT and suggests that miscarriage may result in fewer reported cases.

Disclosure of Interest: None Declared.

Inflammation III

PO329-WED

Association between a pro-inflammatory axis comprised by TSP1-TGFB-CTGF with three micro-RNAs, MIR-19A, 122, and 133A in the pathophysiology of diabetic retinopathy: a therapeutic novel modality

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Background: Diabetic retinopathy (DR) is a severe complication of type-2-diabetes (T2DM). Our laboratory has been studying the role of a pro-inflammatory axis comprised by TSP1-TGFB and CTGF in T2DM. The axis is differentially expressed in patients with T2DM and non-proliferative DR (NPDR) when compared with patients with T2DM and proliferative DR (PDR).

Aims: The purpose of this study was to evaluate selected plasma circulating microRNAs potentially associated with plasma biomarkers including the abovementioned axis and cytokines.

Methods: Institutional IRB and informed consent allowed this prospective study to recruit a total of 23 individuals afflicted by T2DM (n = 10 NPDR and n = 13 PDR) from the Ophthalmology Department Diabetes Clinic at Temple Hospital, normal controls (n = 10) were recruited from the Sol Sherry Thrombosis Research Center at Temple. Multiplexed protein profiling on microarrays by rolling-circle amplification was employed to determine cytokines. Commercially available ELISA was utilized to determine the plasma concentration of TSP1, TGFB and CTGF. Total RNA was extracted from human plasma using the miRCURY™ RNA isolation kit by Exiqon at their facility in Denmark.

Results: TSP1, TGFB, CTGF, IL4, MIP-1β were significantly elevated in NPDR patients when compared to PDR patients. Only three microRNAs were differentially expressed in the normal group when compared with NPDR (decreased), namely miR-133a ($P < 0.005$), miR-19a ($P < 0.008$), and miR-122 ($P < 0.02$) or PDR (decreased), miR-19a ($P < 0.000096$) and miR-122 ($P < 0.005$).

Conclusion: miR-19a has been implicated with expression of TSP1 and CTGF, glucose levels, and insulin resistance. miR-133a has been associated with cardiac hypertrophy, fibrosis and heart failure in diabetes. miR-122 has been identified as a potential biomarker for non-alcoholic fatty liver. In summary our results link for the first time in the literature three circulating microRNAs with the above pro-inflammatory axis as well as the cytokine profile in the pathophysiology of NPDR and PDR.

Disclosure of Interest: None Declared.

PO330-WED

Fibrinogen in sepsis: crosstalk between coagulation and inflammation

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Background: The latest findings have revealed fibrinogen and its cleavage products as the new actors in the innate immune system. Fibrinogen (F1) plays a pivotal role in preventing pathogen invasion and dissemination, as well as, a protective role for the endothelium

via coagulation and fibrinolysis. However, F1 enhances the pro-inflammatory state and if coagulation is exaggerated, it can contribute to disease. Therefore, what is the role of fibrinogen during sepsis?

Aims: The purpose of the study was to evaluate if high F1 levels are associated with survival or non-survival in sepsis.

Methods: We retrospectively analysed 915 adult septic patients, hospitalized within 2000 to 2014 at the University Hospital Innsbruck (Austria). Peak of C-reactive protein (CRP) level was defined as the most intense period of sepsis. CRP and F1 values were collected 3 days before (day -3, day -2, day -1) until 3 days after (day 1, day 2, day 3) peak of CRP level (day 0).

Results: Fibrinogen levels follow the C-reactive protein (CRP) course, though there is no significant difference of CRP-levels between survivor and nonsurvivor. F1 levels in survivors were significantly higher than in non-survivors on each day (day -3 and day -2: $P < 0.05$; day -1: $P < 0.01$; day 0, day 1 and day 2: $P < 0.001$; day 3: $P < 0.01$). In patients with hyperfibrinogenemia survival rate was significantly higher ($P < 0.001$) than in patients with levels within normal range or below. Septic patients with an underlying disease of the central nervous system had higher F1 levels than patients without (day -1 and day 0: $P < 0.01$; day 1, day 2 and day 3: $P > 0.001$) whereas there was no significant difference in F1 levels between survivors and nonsurvivors.

Conclusion: Hyperfibrinogenemia in septic patients is associated with higher survival rate except for patients with a neurological underlying disease where the beneficial role of F1 could not be detected.

Disclosure of Interest: None Declared.

PO331-WED

Increased biomarkers of metabolic syndrome in total joint arthroplasty patients

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Background: Patients undergoing total hip and total knee arthroplasty (THA/TKA) represent a diverse group of individuals with a variety of pre-existing co-morbidities including metabolic syndrome (Mes). Recently several biomarkers of Mes have been identified and may be helpful in the risk stratification and management of arthroplasty patients.

Aims: This study is designed to profile biomarkers of Mes in total joint arthroplasty patients.

Methods: Citrated plasma samples were collected from 42 patients undergoing THA/TKA pre-operatively and day 1 post-operatively (POD1). Control group consisted of 48 healthy, non-smoking and non-medicated donors. Both groups of samples were profiled for Mes biomarkers utilizing a Randox biochip array technology which included C-peptide, ferritin, IL-6, insulin, resistin, TNFα, IL-1α, leptin, and PAI-1.

Results: Compared to normal controls, pre-operative levels of C-peptide and ferritin did not differ significantly ($P > 0.05$). IL-6, resistin, insulin, TNFα, IL-1α, leptin, and PAI-1 levels were significantly higher in the arthroplasty group in comparison to normal controls ($P < 0.05$ to 0.001). Post operative plasma samples exhibited marked augmentation of the biomarkers of Mes with wider variations in comparison to pre-operative levels.

Conclusion: The pre-existing Mes has been reported to contribute to surgical complications in THA/TKA. The increased levels of leptin, insulin, C-peptide, along with inflammatory biomarkers underscore the interplay of Mes mediators and inflammatory process. Profiling of Mes biomarkers provides an added tool to risk stratify arthroplasty patients and optimize their clinical management.

Disclosure of Interest: None Declared.

PO332-WED

End-stage renal disease associated with metabolic syndrome: a study of biomarkers relevant to cardiovascular riskBansal V¹, Saluk J², Hoppensteadt D², Syed D², Abro S², Guler N², Wahi R² and Fareed J²¹Nephrology; ²Pathology, Loyola University Medical Center, Maywood, USA

Background: Metabolic syndrome (MS) represents a cluster of cardiovascular risk factors which contribute to myocardial infarction and stroke in end stage renal disease (ESRD) patients. Several metabolic biomarkers have been identified and their circulating levels provide a better understanding of the pathogenesis of MS/ESRD.

Aims: The purpose of this investigation is to profile biomarkers of MS in ESRD patients.

Methods: Plasma samples from 87 patients with ESRD undergoing maintenance hemodialysis and 50 normals were collected prior to a routine session. These samples were profiled for metabolic biomarkers using multiple protein chip bioarray technology. The protein chip array was comprised of several biomarkers of MS. All results were compiled in terms of group means $\pm$ 1 SD (SEM) and statistically analyzed.

Results: In comparison to the normal samples, the ESRD group showed marked increase in circulating levels of all biomarkers. TNF α (47.4 ± 18.3 vs 4.1 ± 1.1 ng mL⁻¹) and IL-6 (7.8 ± 9.1 vs 0.8 ± 0.4 ng mL⁻¹) showed the most pronounced increase. C-peptide (14.5 ± 4.1 vs 2.8 ± 1.6 ng mL⁻¹), leptin (29.7 ± 29.2 vs 5.2 ± 7.9 ng mL⁻¹), resistin (16.1 ± 6.0 vs 2.3 ± 0.7 ng mL⁻¹) and ferritin (274 ± 57 vs 57 ± 63 ng mL⁻¹) showed a 5-fold increase in the ESRD group compared to normal. PAI-1 (5.4 ± 5.2 vs 2.9 ± 2.5 ng mL⁻¹), IL-1 α (1.3 ± 1.7 vs 0.35 ± 0.07 ng mL⁻¹) and insulin (32.1 ± 17.3 vs 17.1 ± 1.7 ng mL⁻¹) showed modest increase in the ESRD patients. The increase in all of the MS biomarkers in the ESRD patients were highly significantly elevated, $P < 0.05$.

Conclusion: Increase in resistin, insulin, C-peptide, and leptin points to the derangement of glucose metabolism, increased IL-1 α , TNF α , and ferritin suggests the upregulation of an inflammatory process. PAI-1 increase suggests a fibrinolytic deficit. The parallel derangement of inflammatory process, metabolic syndrome and fibrinolytic dysregulation contributes to the cardiovascular and cerebrovascular complications in these patients.

Disclosure of Interest: None Declared.

PO333-WED

Barettin: a natural marine compound with potent anticoagulant and anti-inflammatory propertiesLind KF¹, Olsen JO² and Østerud B³

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Background: Vascular inflammation is a prominent feature of atherosclerotic lesion formation. In search of bioactive products from the sea, we found barettin, isolated from the marine sponge *Geodia baretti*, to possess potent bioactive immunomodulatory effects.

Aims: To study the biological functions of barettin using an ex-vivo whole blood model stimulated with LPS.

Methods: Blood was collected in Fragmin (10 U mL⁻¹). Aliquots were immediately stimulated with LPS (5 ng mL⁻¹) for 2 h at 37 °C in a rotary incubator (190 rpm) in the presence and absence of barettin.

TF activity was measured in isolated frozen and thawed mononuclear cells and plasma samples were assessed for TNF α , MCP-1, IL-10 and high mobility group box protein 1 (HMGB1) by ELISA.

Results: Barettin was recently found by our group to inhibit kinases, in particular receptor-interacting serine-threonine kinase 2 (RIP2) and calcium/calmodulin-dependent protein kinase 1 α (CAMK1 α). Barettin attenuated LPS-induced TF activity and TNF α in a dose-dependent manner. At 5, 10 and 20 μ g mL⁻¹, TF activity was reduced 34.8%, 36.6%, 60.7% and TNF α 35.1, 63.0%, 89.2% ($P < 0.001$). Similarly, LPS-induced MCP-1 (CCL2) was inhibited 46.6%, 55.5% and 46.1% ($P < 0.001$) by barettin at 2.5, 5.0 and 10.0 μ g mL⁻¹. The production of the anti-inflammatory IL-10 cytokine, which preferably should be high, was also reduced by barettin, (45.6%, 54.5% and 34.6% at respectively 2.5, 5.0, 10 μ g mL⁻¹), probably through the inhibition of CAMK1 α . Activated monocytes/macrophages secrete HMGB1 as a cytokine mediator of inflammation. There was some reduction of it by barettin in LPS-stimulated blood but much less pronounced (21.3% at 10 μ g mL⁻¹, non-significant) compared to the other test products. The effect of barettin is currently examined in a mouse model in prevention of lesion formation.

Conclusion: Barettin has a very potent anticoagulant and anti-inflammatory effect that has the potential to prevent inflammatory based diseases including atherosclerosis.

Disclosure of Interest: None Declared.

PO334-WED

Protection effects of 4AAQB isolated from antrodia cinnamomea on lps-induced sepsis modelChang C-H¹ and Chung C-H²

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Background: Sepsis and multiple organ dysfunction syndromes are one of most main causes of death. 4-acetylanthroquinol B (4AAQB) isolated from *Antrodia cinnamomea* (or niu-chang-chih) which have been shown to antioxidation, anti-inflammation and antitumor activities. However, the *in vivo* anti-inflammation effects and detail action mechanism of 4AAQB has not yet been addressed.

Aims: The purpose of this study is to examine whether 4AAQB has the anti-inflammation on sepsis model induced by endotoxin, LPS.

Methods: Mice peritoneal macrophage was used to examine cytokine release, cell migration and signal transduction induced by LPS. In addition, *in vivo* study for cytokine release inhibition and mortality reduction effect were also conducted.

Results: After mice peritoneal macrophage co-treat LPS with different concentration of 4AAQB, the cytokine release induced by LPS was significantly inhibited. The signal transduction induced by LPS including p38, JNK, ERK and NF κ B phosphorylation were significantly inhibited by 4AAQB in mice peritoneal cell. 4AAQB also suppressed LPS-induced mice peritoneal cell migration dose dependently. However, neither co-treatment nor pre-treatment of 4AAQB affect the cell viability.

Conclusion: 4AAQB may inhibit various signaling pathway in LPS-activation macrophages, results in cell migration and cytokine release blockage. These results suggest that 4AAQB may have the therapeutic potential on sepsis.

Disclosure of Interest: None Declared.

PO335-WED

Coagulation activity increases with disease severity in patients with asthma

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Background: Patients with severe asthma are at increased risk of pulmonary embolism as compared to healthy controls, probably due to increased procoagulant and decreased fibrinolytic activity in induced sputum. However, the effect of asthma severity on systemic hemostatic activity is unclear.

Aims: To investigate systemic hemostatic activity in patients with mild, severe and prednisolone dependent asthma as compared to healthy controls.

Methods: In this study, blood samples from baseline were used for a cross sectional analysis between patients with asthma of different severities and healthy controls. Inflammation (C-reactive protein (CRP), leukocytes, neutrophils and eosinophils) and hemostatic parameters (von Willebrand factor (vWF), plasmin-antiplasmin complex (PAPc), plasminogen activator inhibitor type-1 (PAI-1), and endogenous thrombin potential (ETP)) were measured in plasma and compared between the groups. One-way ANOVA with post hoc Bonferroni test was used.

Results: 93 patients with asthma (31 mild, 32 severe, 30 prednisolone dependent) and 33 healthy controls were analysed. Asthma severity was significantly associated with a decrease in forced expiratory volume in one second (FEV1) (overall $P < 0.01$) and increase in blood eosinophils (overall $P < 0.01$). We observed increased vWF, PAPc, and PAI-1 levels with asthma severity (overall $P < 0.01$, $P = 0.04$ and $P = 0.02$, resp.). ETP was higher in patients with prednisolone dependent (126%), severe (119%), and mild asthma (109%) as compared to healthy controls (101%, overall $P < 0.01$). In addition, ETP correlated with peripheral blood leukocytes ($R = 0.3$, $P < 0.01$), neutrophils ($R = 0.3$, $P < 0.01$) and CRP ($R = 0.6$, $P < 0.01$).

Conclusion: Hemostatic activity increases with disease severity in patients with asthma and correlates with neutrophilia in peripheral blood. This suggests that patients with severe asthma are predisposed for pulmonary embolism through inflammation and increased hemostatic activity.

Funding: Dutch Lung foundation, project number 3.2.11.021.

Disclosure of Interest: None Declared.

PO336-WED

Anti-polyphosphate antibodies as anti-inflammatory/ anticoagulant therapeutics

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Background: Inhibitors of polyphosphate (polyP) hold promise as anti-inflammatory or anticoagulant treatments with novel modes of action compared to conventional therapeutics. Monoclonal antibodies have a well-established history as important therapeutics.

Aims: The ubiquity of polyP makes it unlikely that conventional attempts to raise anti-polyP antibodies would succeed. We therefore utilized autoimmune mice (MRL/MpJ-Faspr/J), reasoning that they may generate anti-polyP antibodies as part of their autoimmune disorder.

Methods: We used aged, unimmunized female MRL/MpJ-Faspr/J mice to generate hybridomas, then screened clones for reactivity with polyP in an ELISA. Strongly positive clones were used to generate IgG for further studies. We characterized the ability of the monoclonal antibodies to bind to different sizes of polyP, selectivity for polyP vs. other anionic polymers, and ability to inhibit polyP-mediated coagulation *in vitro*. We also employed antibody PP2059 in mouse models of endotoxemia and hemostasis (tail clip assay).

Results: Anti-polyP antibodies bound mostly to longer chain polyP (>200mer) with varying affinities and specificities for polyP vs. DNA or RNA. Antibody PP2059 inhibited contact activation mediated by long-chain polyP. It was also highly effective in increasing survival in the mouse endotoxemia model, without significantly increasing bleeding in the tail clip model.

Conclusion: Autoimmune mice allowed generation of blocking monoclonal antibodies against polyP that have potential as therapeutics for treating inflammation and coagulation disorders like sepsis, but with reduced bleeding side-effects compared with conventional anticoagulant drugs.

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PO337-WED

Chlamydia infection in venous insufficiency and venous ulcers

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Background: Treatment of venous ulcers (VU) often becomes difficult. Chlamydia (*C.*) induce inflammation and clotting abnormalities. *C. pneumoniae* (*C.pn*) was considered as a source of infection for VU in many studies, but *C. trachomatis* (*C.tr*) wasn't. Both species are common and multiply in epithelial cells and monocytes and can reach any part of organism. *C.tr* induces a significant increase in levels of vascular cell adhesion molecule-1 (CAM1) and mucosal addressin CAM1 taking active part in inflammation.

Aims: Aim of our study was to search role of *C.tr* infection in development of venous insufficiency (VI) in young patients and forming of VU in elders.

Methods: Presence of *C.tr* infection was studied in 3 young patients (2 men, 1 woman aged 32,28,26) with VI (varicose veins and edema) and 7 patients with VU (3 -thrombophlebitis: 1man, 2women aged 50-58; 4-with diabetes type2: 1 man, 3 women, aged 62-74). We used vein blood leukocyte culture method worked out by us, that support proliferation of macrophages in donors and propagation of *C.* in patients with *C.tr* infection that was confirmed by electron microscope studying of culture material (Saralidze, Clinical Atlas of *C.tr* 2005). Culture smears were stained by Giemsa method to detect *C.* inclusions under light microscope and PAS-stain was done to reveal *C.tr* that unlike to *C.pn* consume glucose.

Results: In cultures of all patients were revealed inclusions specific for *C.tr*. Addition of antichlamydial drugs - Azithromycin & Doxycycline in treatment regimen was effective in all cases proved by long remission in all patients and rapid healing of ulcers.

Conclusion: Our study shows that latent *C. infection* plays important role not only in formation of chronic VU, but in the development of VI and not only *C.pn*, but *C.tr* should also be investigated in patients with VU and VI especially in young ones. Blood leukocyte culture method is very useful for this purpose. Inclusion of antichlamydial therapy is reasonable and high effective in treatment regimen of these patients.

Disclosure of Interest: None Declared.

PO338-WED

Early measurement of thrombin activatable fibrinolysis inhibitor (TAFI) and activated TAFI (TAFIA) predict outcome in patients with severe sepsis or septic shock

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Background: Severe sepsis is often associated with coagulation disorders that may precipitate organ failures and death.

Aims: We evaluated the prognostic role of TAFI consumption/activation in patients with severe sepsis or septic shock.

Methods: Three groups of patients were defined in a nested study of the multicenter clinical trial ALBIOS: a) those with normal platelet count at randomization who developed thrombocytopenia during ICU stay (n = 100; 'Worseners'); b) those with low platelet count at randomization (n = 85; 'Positive Controls'); c) those with normal platelet count throughout the study (n = 95; 'Negative Controls', matched for age and sex). TAFI activity, TAFIA(i) antigen (marker of TAFI activation), clot lysis assay, PAI-1, D-dimer, plasmin-antiplasmin complex (PAP), and prothrombin F1 + 2 were measured on day 1 after study entry. The association between biomarkers and mortality was tested with Kaplan-Meier analysis and multivariable Cox models.

Results: Despite normal platelet count on day 1, Worseners had lower TAFI and higher TAFIA(i) than Negative Controls ($P < 0.001$), but levels similar to Positive Controls ($P = \text{ns}$). Clot lysis time, PAI-1 and D-dimer were higher in Worseners and Positive Controls than in Negative Controls, whereas PAP and F1 + 2 were similar in all groups. 90-day mortality was lower in Negative Controls (27.4%), as compared to Worseners and Positive Controls (60.0 and 55.3%, $P < 0.0001$). After adjustment for coagulation-fibrinolysis markers, age, BMI and SOFA score at enrollment, the TAFIA(i)/TAFI ratio, but not PAI-1, remained independently associated with 28-day (HR 1.75 [1.12-2.73], $P = 0.01$) and 90-day mortality (HR 1.84 [1.3-2.9], $P = 0.001$). TAFI consumption and the TAFIA(i)/TAFI ratio predicted incident renal failure ($P \leq 0.04$) and shock ($P \leq 0.01$).

Conclusion: Early TAFI and TAFIA(i) measurements independently predict subsequent organ failure and mortality in patients with severe sepsis or septic shock enrolled in a multicenter clinical trial.

Disclosure of Interest: None Declared.

PO339-WED

Inflammatory cytokine up regulation may be an early marker of thrombosis in patients with implanted LVADs

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Background: The need to predict and prevent left ventricular assist device (LVAD)-associated thrombotic events (occurring most often the first 3 months post-implant) is an unmet clinical need. Inflammation promotes thrombosis by increasing procoagulant factors, inhibiting natural anticoagulant pathways or damaging endothelium.

Aims: To identify changes in levels of markers of inflammation in patients with implanted LVADs associated with clinical adverse events.

Methods: Using the Biochip Array from Randox (Crumlin, County Antrim, UK), inflammatory cytokine levels were quantitated in peri- and post-operative samples collected from 8 patients implanted with a HeartMate II LVAD (Thoratec). Plasmas from 20 healthy individuals established the normal ranges. Adverse events were determined from medical chart review.

Results: Pre-surgery, most patients exhibited IL6 and IL8 levels above normal. Post-LVAD implantation, the levels of IL6, IL8, TNF α and IL1 β were further increased. The systemic inflammatory response lasted for 2 months post-surgery. In some patients, IL6 and IL8 were several hundred-fold higher than normal for 6 months. In patients with adverse events IL2, IL4, IL6, IL8, TNF α , and IL1 β were excessively elevated. Levels of cytokines whose function is more closely tied to antigen-mediated immunity were not significantly impacted by LVAD implantation.

Conclusion: There is a pattern of increasing cytokine up regulation with increasing medical concerns. The variability in cytokine levels among patients and the high peak levels of IL6 and IL8 at various time points suggest isolated pathologic activation unique to each LVAD implanted patient. These data are being analyzed in conjunction with thrombelastography, platelet activation and cellular microparticle data to identify relationships that associate with clinical events. This study will provide supportive evidence to identify LVAD-associated mechanism(s) of thrombosis and clinically useful biomarkers.

Disclosure of Interest: None Declared.

PO340-WED

Annexin A1 concentration is increased in patients with preeclampsia

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Background: Preeclampsia (PE) is characterized by endothelial dysfunction and inflammation. Annexin A1 (AnxA1) is an endogenous anti-inflammatory and pro-resolutive protein that has not been studied in PE.

Aims: To investigate protein and transcript levels of AnxA1 in PE women and the association among AnxA1, C reactive protein (CRP), soluble endoglin (sEng) and soluble tumor necrosis factor alpha receptor type 1 (sTNF-R1) plasma levels.

Methods: This study included 40 non-pregnant, 38 normotensive pregnant (< 34 weeks of gestation: n = 14; $\geq$ 34 weeks of gestation: n = 24) and 51 PE women (early: n = 23; and late: n = 28 clinical

forms). AnxA1 protein levels in the plasma and transcript levels in peripheral blood mononuclear cells (PBMC) were determined by ELISA and real time RT-PCR, respectively. CRP plasma levels were measured by immunoturbidimetric ultrasensitive assay. This study was approved by the Ethical Committee of Universidade Federal de Minas Gerais (protocol number 0618.0.203.000-10), and informed consents were signed by the participants.

Results: AnxA1 and CRP plasma levels were increased in PE women. The early PE subgroup had higher levels of AnxA1 and CRP than the respective pregnant control, while no significant difference was detected comparing late PE and the respective control. AnxA1 and CRP levels were similar between early and late PE. AnxA1 mRNA levels were not significantly different among the study groups. AnxA1 was positively correlated with sEng and sTNF-R1, but not with CRP. CRP was positively correlated with sEng and sTNF-R1.

Conclusion: AnxA1 increase in PE women points to a possible dysfunction of this resolution pathway, since this event is unable to resolve the systemic inflammatory response, an important feature of PE. Further studies are needed to test this hypothesis. The association between inflammation and endothelial dysfunction was confirmed by the correlation analyses.

Financial support: FAPEMIG, CNPq and PRPq-UFG.

Disclosure of Interest: None Declared.

PO342-WED

Combined high levels of IL-18 and IL-12 are associated with new clinical events in CAD patients

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Background: The pro-inflammatory cytokine Interleukin-18 (IL-18) in synergy with Interleukin-12 (IL-12) is critical in the initiation and progression of T helper cell 1- type responses, characterized by cytokine and chemokine production. Elevated levels of both markers have been shown associated with atherosclerosis. Their regulation/production and interaction have been hypothesized to be driven by glucose.

Aims: To explore the possible role of simultaneously elevated levels of IL-18 and IL-12 in presence or absence of high glucose, for the prognosis in coronary artery disease (CAD).

Methods: Patients (n = 1000) with angiographically verified stable CAD were investigated (220 type-2 diabetics, 203 current smokers, 78% men, mean age 62 years). Informed consent was obtained from all and the study was approved by the Regional Ethics Committee. IL-12 and IL-18 were measured by ELISA methods. High fasting glucose (FG) was defined as > 5.6 mmol L⁻¹.

Results: After 2 years follow-up, 106 clinical endpoints, defined as a composite of acute myocardial infarction, unstable angina, stroke and death, were recorded. IL-12 levels were significantly lower in current smokers ($P < 0.001$) and the results were stratified accordingly. High FG associated with higher levels of IL-12 and IL-18, of which the elevated IL-18 levels associated with clinical events in non-smokers ($P = 0.012$, adjusted for age and gender). At low FG, we observed no significant associations to clinical endpoints, irrespectively of smoking or not. When dividing IL-12 and IL-18 levels into tertiles (Ts), dichotomized between T1 and T2/T3, subjects simultaneously in the two upper Ts of both markers were at higher risk of clinical events, exclusively in non-smokers (OR=1.6, 95% CI 1.0, 2.6, $P = 0.04$, adjusted), and independent of FG levels.

Conclusion: Cigarette smoking seems to abolish the significantly elevated IL-18 levels induced by high FG. Combined elevated levels of IL-12 and IL-18 increase the risk of clinical events in non-smokers.

Disclosure of Interest: None Declared.

PO343-WED

Determination of heparin level in blood plasma – sensitivity and specificity as a marker for identification of systemic mast cell activation disease

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Background: The disorders *idiopathic systemic mast cell activation syndrome* (MCAS) and *systemic mastocytosis* (SM) are summarized under the generic term *systemic mast cell activation disease* (MCAD). MCAD is characterized by an enhanced release of mast cell-derived mediators inducing various mediator-related symptoms. The diagnosis of MCAD usually requires demonstration of increased mast cell mediator release. However, only a few mast cell mediators are currently established as biomarkers of MCAD.

Aims: Examination of the sensitivity of plasma heparin level as indicator for an increased mast cell activation in comparison with that of serum tryptase and chromogranin A (CgA) and N-methylhistamine in a large cohort of MCAD patients (n = 285).

Methods: For determination of plasma heparin levels blood sampling was done using CTAD Monovette™ tubes (Sarstedt, Nuembrecht, Germany) and cooled to +4°C until centrifugation in the cold. Plasma heparin level was measured by chromogenic anti-factor Xa assay (COAMATIC Heparin, Chromogenix, IL, Milano, Italy).

Results: Basal plasma heparin level had a sensitivity of 41% in MCAS patients and 27% in SM patients. Mild non-pharmacologic stimulation of mast cell degranulation by obstruction of venous flow for 10 min increased the sensitivity of plasma heparin level in MCAS patients to 59% and in SM patients to 47%. In MCAS patients tryptase, CgA and N-methylhistamine levels exhibited low sensitivities (10%, 12% and 22%, respectively), whereas sensitivities for SM were higher (73%, 63% and 43%, respectively).

Conclusion: Taken together, these data suggest that plasma heparin level appears more sensitive than the other mediators for detecting systemic mast cell activity in patients with MCAS. The increase in plasma heparin levels following brief venous occlusion of the arm appears to be a rough indicator of the degree of irritability of the pathological mast cell population in MCAD.

Disclosure of Interest: None Declared.

PO344-WED

Cytokine-mediated triggering of thrombin generation and inflammation in patients with Stevens-Johnson syndrome/toxic epidermal necrolysis

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Background: Stevens-Johnson Syndrome and its more severe variant, toxic epidermal necrolysis is a rare, most commonly drug-induced, acute, exfoliative disease involving the skin and mucous membranes. Major Histocompatibility Complex-restricted presentations of the culprit drug triggers T-cell activation that causes expression of TNF- α , interferon- γ , and granzyme B from Natural Killer cells.

Aims: It is hypothesized that these cytokines trigger thrombin generation and inflammation.

Methods: Blood samples were obtained from subjects suspected of SJS/TEN. Diagnosis based on histopathology was confirmed in 4 and unconfirmed in 7 patients. Cytokine levels were measured using the Cytokine High Sensitivity biochip arrays. Thrombin-antithrombin complexes (TAT), prothrombin fragment (F1.2), PAI-1, IL-6, Protein C, MCP-1, Endocan and microparticles were measured using ELISA methods. Antithrombin levels were measured using an amidolytic method.

Results: IL-6 ($P = 0.0138$), and MCP-1 ($P = 0.0166$) levels were increased significantly in biopsy-confirmed SJS-TEN when compared to normal. A marked increase in the TAT complexes ($6.3 \pm 5.9 \mu\text{g mL}^{-1}$), F1.2 ($430.4 \pm 202.4 \text{ pmol L}^{-1}$), platelet microparticles ($13.1 \pm 9.3 \text{ nM}$) and protein C levels ($90.5 \pm 63.4\%$) with a corresponding decrease in PAI-1 ($53.3 \pm 18.8 \text{ ng mL}^{-1}$) and antithrombin levels ($80.7 \pm 42.4\%$) were also observed. Biopsy negative SJS/TEN subjects demonstrated mild elevation in cytokine levels of IL-6 ($29.81 \pm 25.18 \text{ pg mL}^{-1}$), TNF- α ($7.20 \pm 5.04 \text{ pg mL}^{-1}$) and MCP-1 ($265.10 \pm 159.09 \text{ pg mL}^{-1}$). Endocan levels were significantly elevated in SJS patients when compared to the normal ($P < 0.05$).

Conclusion: Cytokine-mediated triggering of thrombin generation and inflammation resulted in an increase in TAT, MCP-1, F1.2 and platelet microparticles and corresponding decrease of protein C, antithrombin, and PAI-1 levels may lead to adverse sequelae such as DIC. The increased levels of endocan are consistent with elevation of other biomarkers of inflammation.

Disclosure of Interest: None Declared.

Management of bleeding II

PO345-WED

Nonclinical evaluation of cationically modified polysaccharide antidotes for unfractionated heparin

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Background: Although many new antithrombotic agents were introduced in the last few years, unfractionated heparin (UFH) remains a key drug inhibiting blood coagulation in case of emergency. Because of bleeding risk, UFH therapy requires continuous monitoring and neutralization with suitable antidotes. Protamine, the only clinically approved antidote to UFH, may produce serious adverse effects.

Aims: We aimed to develop an alternative synthetic UFH antidote as efficient as protamine, but safer and easier to produce.

Methods: Our approach was to synthesize, purify and characterize cationic derivatives of non-toxic, biocompatible, widely available, inexpensive, and easy to functionalize polysaccharides: dextran, hydroxypropylcellulose, pullulan and γ -cyclodextrin. We screened them for potential heparin-reversal activity using a colorimetric method with Azure A as the indicator and we examined efficacy and safety of the most active polymers in Wistar rat model of experimentally induced arterial thrombosis. Efficacy studies included the measurement of thrombus formation, activated partial thromboplastin time (aPTT), bleeding time, and anti-factor Xa activity; safety studies included the measurement of hemodynamic and hematologic parameters.

Results: UFH decreased thrombus weight (by 38%; $P < 0.001$), prolonged aPTT (14 times; $P < 0.001$) and bleeding time (by 60%; $P < 0.001$), and increased anti-factor Xa activity (7 times; $P < 0.001$). Only linear, high molecular weight dextran substituted with glycidyltrimethylammonium chloride groups at a ratio of 0.65 per glucose unit (Dex40-GTMAC3) restored all parameters to control levels without changing of hematological and hemodynamic values.

Conclusion: Dex40-GTMAC3 is an easy to synthesize, efficient and safe heparin antidote that could replace protamine in the treatment of heparin associated bleeding.

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Disclosure of Interest: None Declared.

PO346-WED

Screening tool for bleeding disorders in women with heavy menstrual bleeding

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Background: Women with heavy menstrual bleeding (HMB) have an increased risk of bleeding disorders. It would be efficient and decrease costs if one could predict who should be tested for bleeding disorders. Previous screening tools were not sufficiently specific.

Aims: To develop a screening tool for bleeding disorders in women with heavy menstrual bleeding.

Methods: After informed consent we included 205 consecutive women with regular HMB (Pictorial Bleeding Assessment Chart-score >100). In all women gynecological examination was performed, blood was drawn (blood count, thyroid function and haemostatic parameters) and a questionnaire was filled out (containing Tostetto bleeding score). A bleeding disorder was defined as decreased fibrinogen, decreased von Willebrand factor, and/or factor VIII, IX, XI deficiencies. Development of the screening tool: we performed forward selection for each covariate by univariable logistic regression. Covariates selected for multivariable model were significant ($\alpha < 0.15$) or considered as essential. For the screening tool each covariate was given a certain number of points based upon the odds ratio in the model.

Results: We identified 11 (5%) women with a bleeding disorder. Anemia (3 points), family member with bleeding disorder (3), easy bruising (2), HMB since menarche (1) and bleeding after procedure (1) were the 5 out of 24 variables we identified for our screening tool. With a cut-off of ≥ 1 point, sensitivity was high (92%) with acceptable specificity (52%), reducing the proportion needing to be tested with 49%. Negative likelihood ratio (LR) was 0.18. Alternatively, the tool could be used to identify women with a high probability of bleeding disorders by using a cut-off of ≥ 4 points (sensitivity: 36%, specificity: 93%, LR+: 5.43).

Conclusion: We developed a simple screening tool, consisting of 5 variables, that reliably excludes bleeding disorders in women with heavy menstrual bleeding. This tool needs to be validated in other groups of women with HMB.

Disclosure of Interest: None Declared.

PO347-WED

The impact of red blood cells on hemostasis under whole blood flow conditions

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Background: Red blood cells (RBC) are the essential factor in hemostasis. Under blood flow conditions, RBC are considered to promote platelet-collagen interaction rheologically by transporting platelet towards vessel walls. We recently experienced a clinical case with vWD who remarkably improved after RBC transfusion. We also monitored the hemostatic status of this case using the microchip flow chamber system, named T-TAS (total thrombus analysis system). **【Patient's profile】** A 14-year-old girl who was diagnosed as type 1 von Willebrand disease (VWD) presented with anemia due to hypermenorrhea.

Even though VWF:RCO had increased >100% with the replacement therapy with FVIII/VWF, the bleeding aggravated and thrombus formation was disabled in T-TAS. After RBC transfusion, however, bleeding stopped and T-TAS was improved. Therefore, we speculated that RBC was required to stop bleeding.

Aims: To analyze the impact of RBC on hemostasis, the reconstituted blood of varying RBC concentrations were evaluated comprehensively.

Methods: In T-TAS, pressure inside the microchip flow chamber coated with collagen (platelet(PL)-chip) for 10 min or collagen/TF (Atherome(AR)-chip) for 30 min during the perfusion of whole blood was monitored. The rate of flow was 1000 or 2000s-l in PL chip, while it was 240s-l in AR-chip. Reconstituted blood containing different hematocrit (Ht: 0, 10, 25, 50%) of RBC were tested. T₁₀ or T₃₀ (time to 10 or 30 kPa) were evaluated. The effect of Ht was also analysed in Rotational thromboelastometry (ROTEM) and Multiplate.

Results: In T-TAS, T₃₀ with AR-chip delayed with the decrease of RBC(13.1, 17.8, 17.5, 22.5 min). T₁₀ with PL-chip(1000s-l) delayed with the decrease of RBC(2, 6.5, 10, 10 min). Furthermore, this delay of occlusion time was more apparent at 2000s-l(2, 10, 10, 10 min). By contrast, no difference was observed in ROTEM and Multiplate.

Conclusion: The important role of RBC on hemostasis under flow conditions was confirmed both *in vivo* and flow-based *in vitro* system.

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PO348-WED

Reversal of dabigatran could be achieved by hemodialysis with or without other hemostatic agents

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Background: Patients may develop life-threatening bleeding during dabigatran therapy, which requires rapid reversal of its anticoagulant effects. Although specific antidotes are being evaluated in clinical trials, none are yet available for routine use. Consequently, hemodialysis (HD) or non-specific hemostatic agents such as recombinant factor VIIa (rFVIIa), or Factor Eight Inhibitor Bypass Activity (FEIBA) may be employed for the control of hemorrhage.

Aims: To summarize literature data from human case report discussing the reversal of dabigatran using HD.

Methods: A literature search was conducted using the OVID Medline database, from Jan 2000 to Aug 2014 using the keywords 'dabigatran' and 'reversal'. The search was limited to human studies written in English. Only publications containing patients' clinical information were included for data extraction. The citations in the included publications were scanned for any missing articles in our original list. The duration to stop bleeding after intervention(s) were compared between the two patient groups: 1) the patients undergoing HD only; 2) those receiving other hemostatic agents along with HD. Statistical analysis was performed using the Student's *t*-test. A *P* value < 0.05 was considered significant.

Results: Of the initial 2552 articles retrieved from the Medline database, 12 articles contained sufficient information for review. There were a total of 11 bleeding patients on dabigatran, who survived after HD; 5 were on HD alone, and 6 received rFVIIa in addition to HD. No patients received FEIBA in addition to HD. Six patients had fatal outcomes, mostly due to multi-organ failure or disseminated intravascular coagulopathy. Patients receiving only HD required an average of 16.5 h to stop bleeding whereas those treated with HD and rFVIIa took 14 h (*P* = 0.4).

Conclusion: Although the number of patients in the literature is limited, hemostasis has been successfully achieved using either HD alone, or in concert with rFVIIa, in bleeding patients on dabigatran.

Disclosure of Interest: None Declared.

PO349-WED

Increased coagulation and fibrinolytic potential of solvent-detergent plasma: an observational study between SD-treated pooled plasma and fresh frozen quarantine plasma

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Background: The administration of plasma is widely used in clinical practice, although administration of plasma is not completely devoid of risks. To overcome these potential life-threatening drawbacks solvent-detergent treated plasma (SD-treated plasma) was developed in The Netherlands.

Aims: The aim of this study was to examine the potential differences in fibrinolysis and coagulation potential between single donor quarantine plasma (Q-plasma) and SD-treated Omniplasma. Thawing time as well as the stability of coagulation proteins was studied after storage at 2-6°C.

Methods: 10 Omniplasma's (3 batches), and 10 single donor Q-plasma's were used to study coagulation (APTT, PT, factor V, VII, VIII, XI, XIII, vWF and thrombin generation), anticoagulation (free Protein S and antithrombin (AT) in complex with thrombin, factor IXa or factor Xa) and fibrinolytic (ROTEM tPA lysis time, α 2-antiplasmin, PAI-1 and plasminogen) parameters. Analysis was performed from baseline up to 72 h of storage at 2-6°C after thawing.

Results: At baseline, significant reduced levels of factor V, free protein S, α 2-antiplasmin and tPA-induced ROTEM lysis time were observed in Omniplasma as compared to all single donor quarantine plasma units. Moreover, thrombin generation and IXa-AT complex are significantly increased in all Omniplasma batches. The majority of the parameters studied remained stable in Omniplasma 48 and 72 h after thawing, with the exception of vWF, factor XI and IXa-AT.

Conclusion: Our results suggest an increased coagulation potential, possible as a result of contact activation and on the other hand, an increased fibrinolytic potential in Omniplasma. The stability of Omniplasma, based upon the different parameters studied is comparable to Q-plasma. A storage time of 48 or 72 h after thawing for Omniplasma can be suggested.

Disclosure of Interest: None Declared.

PO350-WED

Prospective Hemostasis Registry of Patients in Cardiac Surgery (HEROES-CS): evaluation of the introduction of a thromboelastography based transfusion protocol in cardiac surgery

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Background: The use of transfusion and coagulation management algorithms guided by thromboelastometry has been associated with reduced blood transfusion requirements, reduced transfusion-related adverse events and improved outcomes in cardiac surgery.

Aims: To prospectively evaluate the impact of ROTEM based procedures on cardiothoracic surgery/ICU (use of coagulation factor concentrates, transfusion requirements, laboratory orders and patients outcomes).

Methods: Consecutive patients in two fixed periods of time, undergoing different types of cardiac surgery were included. The first group (n = 199) was transfused using a transfusion protocol based on traditional coagulation tests (PT, aPTT, fibrinogen, platelet count). The second group (n = 137) was included after a long transition period in which a ROTEM transfusion protocol was introduced and staff was trained. All patients were prospectively followed during surgery and ICU stay.

Results: Patient characteristics and surgery types were similar in both groups. Surgery duration, clamp time and on extracorporeal bypass were significantly longer in the ROTEM group. The mean number of transfusions per patient was reduced for all blood types in the ROTEM period (RBCs from 1.17 to 0.50, FFPs from 0.55 to 0.09 FFP, PCs from 0.22 to 0.09 units per patient). The % of patients receiving transfusions was reduced as well. Re-explorations for postoperative bleeding /tamponade reduced from 9.5% to 4.4%. Peri-operatively the % of patients receiving fibrinogen showed an increase, from 1.5% to 19.7% in the ROTEM group. Desmopressin use decreased and Novo 7 use was negligible in both periods (n = 1). Ordering of ROTEM tests increased significantly (mean 4.4 tests per patient) while ordering of traditional coagulation laboratory tests remained stable.

Conclusion: Introduction of a ROTEM based protocol changed transfusion policy from RBCs, FFPs and PCs to fibrinogen. The number of ROTEM tests increased whereas traditional laboratory tests were still ordered.

Disclosure of Interest: None Declared.

PO351-WED

Side effects of and response to ddavp do not correlate with overweight in patients with hemophilia A or von Willebrand disease

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Background: DDAVP is used to improve hemostasis in patients with mild hemophilia A (HA) or von Willebrand disease (VWD). It releases endogenous von Willebrand Factor (VWF) and increases factor VIII. DDAVP response and occurrence of side effects differ between patients. DDAVP is currently dosed based on total body weight (TBW). It is not known whether overweight may lead to overdosing.

Aims: To assess if body weight is related to side effects and response to DDAVP.

Methods: Two cohorts of patients that received DDAVP in a dose of 0.3 µg kg⁻¹ intravenously, were included: HA patients in whom retrospective response data were available and patients with HA or VWD type I in whom prospectively both response data and side effects were collected. Informed consent was obtained. The patients were divided into groups based on body mass index (BMI), ideal body weight (IBW) ratio (TBW/IBW) and total dose of DDAVP. Response was measured as fold increase in VWF after DDAVP. Side effects were evaluated by change in systolic blood pressure, heart rate and plasma sodium concentration before and directly after, as well as 6 and 24 h after DDAVP administration. The study was exempt from review by a medical ethics committee.

Results: Data of 136 patients were analyzed, 54% of whom had VWD. Of these VWD patients, 74% was female. Age ranged from 16 to 75 years (median 33 years). Median TBW was 75 kg (IQR 67-88 kg) and median height 1.76 m (IQR 1.68-1.82 m). Overweight (BMI > 30 and/or IBW ratio >1.25) was seen in 38 patients. No correlation was

found between the DDAVP induced increase of VWF and BMI, IBW ratio or total dose of DDAVP. Hypotension and tachycardia were not more frequently observed in overweight patients or patients with a DDAVP dose > 20 µg (N = 79) compared to normal weighted patients. Occurrence of hyponatremia was not correlated with BMI, IBW-ratio or total DDAVP dose.

Conclusion: DDAVP dosing based on total body weight does not result in more side effects or a better response in overweight patients with HA or VWD.

Disclosure of Interest: None Declared.

PO352-WED

Thrombo(Elasto)graphic evaluation of the influence of anticoagulant constituents on the prohemostatic potential of prothrombin complex concentrate and factor IX concentrate

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Background: Coagulation factor concentrates like factor IX (FIX) and prothrombin complex concentrate (PCC) can contain anticoagulant substances that may hamper their procoagulant effectiveness in the treatment of haemophilia B or reversal of oral anticoagulation, as well as the laboratory assessment thereof.

Aims: To compare composition and prohemostatic effectiveness of different PCCs and FIX concentrates.

Methods: Composition was assessed by classical laboratory tests and mass spectrometry. Prohemostatic effectiveness was evaluated in vitro employing thrombography (TGA) and thromboelastography (TEG) with FIX deficient plasma, vitamin K antagonist (VKA)-anticoagulated plasma and plasma anticoagulated with rivaroxaban. Coagulation was triggered with low dose tissue factor (1-10 pM).

Results: FIX was the major component in all examined FIX concentrates. In contrast, in all PCCs and apart from vitamin K-dependent coagulation factors, an abundant amount of general plasma proteins were detected. Most PCCs contained antithrombin or heparin, while heparin was detected in 1 out of 4 examined factor IX brands. Recombinant factor IX showed a slightly increased prohemostatic activity compared to plasma derived products that may relate to the presence of activated FIX. All unfractionated heparin (UFH)-containing clotting factor concentrates (0.04-0.40 IU UFH/IU FIX) showed severely hampered prohemostatic effects when therapeutic doses were added to anticoagulated plasmas. This may be explained by the high sensitivity of both TGA and TEG for UFH (IC₅₀ ± 0.05 IU mL⁻¹). Upon heparinase treatment, comparable prohemostatic effects were observed.

Conclusion: Compositional differences between clotting factor concentrates, especially the presence of heparin, may translate into unrealistic laboratory results in treated patients. Heparin in clotting factor concentrates may become clinically relevant at high prohemostatic drug doses, particularly those recommended in the reversal of non-VKA anticoagulants such as rivaroxaban by PCC.

Disclosure of Interest: H. J. Brinkman Employee of: Sanquin Blood Supply Foundation, manufacturer of clotting factor concentrates, S. Patiwaal Employee of: Sanquin Blood Supply Foundation, manufacturer of clotting factor concentrates, S. Tripathi Employee of: Sanquin Blood Supply Foundation, manufacturer of clotting factor concentrates, A. Meijer Employee of: Sanquin Blood Supply Foundation, manufacturer of clotting factor concentrates, J. Meijers Employee of: Sanquin Blood Supply Foundation, manufacturer of clotting factor concentrates.

PO353-WED

Prothrombin complex concentrate can increase thrombin generation in the presence of low platelet number

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Background: While platelet transfusion remains the treatment of choice for prevention and treatment of thrombocytopenia associated bleeding, we speculated that increasing the concentration of procoagulant factors by giving prothrombin complex concentrate (PCCs) in the presence of low platelet numbers may increase thrombin generating potential and contribute to normalising haemostasis.

Aims: To evaluate the effect of PCCs (prothrombin complex concentrates) on thrombin generation in an *in vitro* model of thrombocytopenia.

Methods: Autologous plasma was used to prepare platelet rich plasma (PRP) over a range of platelet counts (20, 40, 60, 90, 150, 300 × 10⁹/L) (n = 5, normal donors). The PCC used in this study contains factors II, VII, IX, X, and proteins C and S. At each platelet count, PRP thrombin generation (0.5pM tissue factor trigger) was measured at baseline and after the addition of 0.3 and 0.6 IU mL⁻¹ PCC with prothrombin as reference protein, (equivalent to 10 to 15, 25 and 50 IU kg⁻¹) using the calibrated automated thrombogram.

Results: Spiking with PCC resulted in an increase in peak IIa (nM) and rate (nM/min) of thrombin (IIa) generation that was both dose and platelet count dependent when compared to baseline levels (n = 5). When baseline peak IIa generation was compared between the lowest and highest PRP platelet counts tested (20 vs 300), mean peak IIa concentrations were 61.6 nM (SEM= 8.8) and 102.7 nM (SEM = 15.2) respectively. Addition of 0.3 IU mL⁻¹ PCC (20 × 10⁹ L⁻¹) increased the mean peak to 78.6 nM (28%, SEM=10.1) while mean peak after the addition of 0.6 IU mL⁻¹ (185 nM, 200%, SEM = 26) exceeded the baseline peak at 300 × 10⁹/L by 45%.

Conclusion: Addition of a therapeutic dose of PCC to PRP at a platelet count that coincides with severe thrombocytopenia increases the peak and rate of IIa generated to those of normal platelet numbers inferring a potential for therapeutic application.

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PO354-WED

Evaluation of activated prothrombin complex concentrate use and its role in the reversal of novel oral anticoagulants

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Background: Novel oral anticoagulants dabigatran, rivaroxaban, and apixaban have become increasingly popular despite limited clinical data for an effective reversal agent. Activated prothrombin complex

concentrate (aPCC) may have a place in therapy for reversing major bleeding events associated with these medications.

Aims: The study objectives are to characterize aPCC use in novel oral anticoagulant-related bleeding and evaluate adherence to institutional guidelines.

Methods: This is a single-center, retrospective review of patients who received aPCC at our institution from January 1, 2013 through August 31, 2014 for rivaroxaban, dabigatran, or apixaban reversal. Data was analyzed using descriptive statistics.

Results: Twenty-nine patients were treated with aPCC. The most common indication for anticoagulation was atrial fibrillation. Anticoagulation medications were dosed inappropriately based on renal function in approximately one quarter of patients, and concomitant antiplatelet therapy was prescribed in over half of patients. The mean aPCC dose was 52.5 ± 13 units kg⁻¹. Twenty-four patients (83%) met International Society on Thrombosis and Haemostasis (ISTH) major bleed criteria, and one patient required aPCC for an emergent surgery. Intracranial bleeding was the most common indication for aPCC administration, followed by gastrointestinal bleeding. Five patients (17%) experienced thrombotic events post-aPCC administration, with only one being within 48 h of aPCC administration. Twenty-one patients (72%) survived to discharge, with 15 patients discharged to sub-acute care facilities.

Conclusion: Use of aPCC at our institution was consistent with the approved institutional guidelines. Most patients survived to discharge, indicating that aPCC may play a role in reversal until specific antidotes are available. Larger randomized trials are needed to determine the efficacy and safety of aPCC for reversing novel oral anticoagulants.

Disclosure of Interest: None Declared.

PO355-WED

The cost-effectiveness of PD-APCC vs. RFVIIa in pediatric patients with haemophilia A and inhibitors with mild to moderate joint bleeding in MEXICO

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Background: Activated prothrombin complex concentrate (pd-aPCC) and recombinant factor (rFVIIa) are two bypassing agents that close gaps between inhibitor and non-inhibitor patients receiving clotting factors by providing better bleeding resolution rates.

Aims: This study compares the cost-effectiveness of rFVIIa vs. pd-aPCC in pediatric patients with haemophilia and inhibitors with mild-to-moderate joint bleeding in Mexico.

Methods: A decision tree to assess the costs and effects of treating these patients with rFVIIa and pd-aPCC in Mexico was developed using TreeAge 2014. The model takes the perspective of the Mexican public healthcare payer. Clinical and local cost data were collected from literature review (Treur et al 2009) and official sources. The data was validated and a maximum time horizon was set as 7 days by local Haemophilia experts (time where all joint bleeds resolve if not by drug then spontaneously). The base case assumes a patient with a weight of 30 kg. Sensitivity analyses on weight, time horizon and other key drivers (efficacy and cost) were performed. Patient weight was studied between 10 and 50 kg.

Results: At the 7th day, rFVIIa provides savings of MXP12,981 compared to pd-aPCC in pediatric patients of 30 kg (MXP34,307 and MXP47,288 with rFVIIa and pd-aPCC, respectively). At the 2nd day (time where most joint bleeds resolve), rFVIIa is dominant with a positive incremental bleeding resolution rate of 0.08 and a cost per patient of MXP16,680 compared to MXP25,732 for pd-aPCC. Treatment dosage and bleeding resolution rates are the main drivers of the model.

Weight has an impact on drug costs and savings achieved. At day 7, savings may vary between MXP12,981 and MXP48,234.

Conclusion: The economic analysis demonstrated savings with rFVIIa in the treatment of pediatric patients with haemophilia and inhibitors with mild to moderate joint bleeding compared to pd-aPCC. The savings provided by rFVIIa result of an earlier resolution of bleeds.

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PO356-WED

Platelet ADP reactivity and bleeding risk in long-term double antiplatelet therapy patients with coronary artery disease

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Background: Platelet dysfunction is a common cause of bleeding (BL) throughout the long-term double anti-platelet therapy (DAPT). The occurrence of even minor («nuisance») BL in patients may result in premature drug discontinuation and impact negatively the clinical outcome.

Aims: To explore platelet reactivity to ADP in long-term DAPT coronary artery disease (CAD) patients manifesting minor (BARC Type 1) BL.

Methods: Total of 115 CAD pts (76% males, avg. age 61 ± 10.7 years) on long-term DAPT (aspirin 100 mg day⁻¹ and clopidogrel 75 mg day⁻¹) were included into the study. Mean follow-up period was 1.09 ± 0.3 years. BL events have been characterized by BARC-defined BL classification. Platelet reactivity to ADP was measured on VerifyNow P2Y12 (Accumetrics, San Diego, CA).

Results: BARC Type 1/2/3-5 BL have been manifested in 57 (49.6%) / 12 (10.4%) / 4 (3.5%) patients. The optimal threshold value of platelet ADP reactivity in Type 1 BL was set at 205 PRU. The Odds Ratio of Type 1 BL (adjusted to age, gender and clinical factors) in patients with platelet ADP reactivity value < 205 PRU was 7.8 (95% CI, 2.6-23.3; $P = 0.0002$). Patients with Type 1 BL revealed the significantly lower incidence of stent thrombosis and recurrent PCI than other patients: 2.08% vs 9.78% ($P = 0.02$), respectively. Patients with Type 1 BL possessed the similar incidence

of major Type 3-5 BL comparing to other patients: 3, 13% vs 3, 23% ($P = \text{NS}$), respectively.

Conclusion: BARC Type 1 («nuisance») bleedings are the most common in long-term DAPT patients. Platelet ADP reactivity value < 205 PRU could be considered as a reliable measurable parameter for the prediction of BARC Type 1 BL in long-term DAPT patients.

Disclosure of Interest: None Declared.

PO357-WED

Thrombin activatedfibrinolysis inhibitor is a significant risk factor of recurrent bleedings in patients on long-term warfarin therapy

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Background: Recurrent bleedings are the most frequent and serious complications in long-term warfarin (W) therapy patients.

Aims: The current study was performed to examine the plasma levels of thrombin-activatable fibrinolysis inhibitor (TAFI) as a potential prognostic risk factor for the recurrent bleedings in long-term W therapy patients.

Methods: Total of 150 atrial fibrillation or venous thromboembolism patients (80 males, aged 64.7 ± 11.2 years) long-term treated with W have been recruited to the study. All patients have been monitored in coagulation clinic where PT and INR have been examined monthly. The follow up period lasted for 3.1 ± 3 years. TTR exceeded 60%. TAFI plasma levels were assayed spectrophotometrically with STACHROM TAFI reagent kit.

Results: Half of the patients ($n = 75$) revealed the haemorrhagic complications. Specifically, 46 patients (61.3%) had recurrent bleedings and 29 patients (38.7%) had single bleeding. Since TAFI levels distribution was nonparametric the quartile analysis was applied. TAFI level spread from 47% to 228%. Distribution median is 103.2%, interquartile range is 90% up to 120%. The clinical and laboratory factors with $P < 0.2$ were identified by univariate analysis and then screened by discriminant function analysis. Subsequently, the following predictors for the recurrent bleedings have been verified: history of bleeding (F-remove=13.4; $P = 0.0003$), labile INR (F-remove=9.02; $P = 0.0031$), triple antithrombotic therapy (F-remove=4.69; $P = 0.031$), and 1-3 quartiles of TAFI distribution (F-remove=5.63; $P = 0.018$).

Conclusion: Along with the well-characterized risk bleeding factors such as bleeding history, labile INR and triple antithrombotic therapy our study suggests that the plasma TAFI levels less than 120% could be considered as an additional risk factor for the recurrent bleedings in long-term W therapy patients.

Disclosure of Interest: None Declared.

PO358-WED

Low dose FEIBA and hemodialysis for managing major bleeding on Dabigatran

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Background: Dabigatran is a direct thrombin inhibitor primarily renally eliminated and removable by hemodialysis (HD). Studies exploring the pharmacokinetics of dabigatran in end stage renal disease and impact of HD used a single dose model, capturing drug primarily in the plasma and not tissue distribution, resulting in overestimating dabigatran removal. We previously reported a case using a low dose (8 units kg⁻¹) of the activated prothrombin complex concentrate (aPCC) administered immediately prior to the successful insertion of

the dialysis catheter with subsequent HD and post dialysis plasma dabigatran concentration rebound.

Aims: This retrospective analysis included patients who required emergent HD to remove dabigatran with low dose FEIBA (1 vial ~ 8 units kg⁻¹) for dialysis catheter placement. HD was subsequently initiated and dabigatran management assessed.

Methods: Dabigatran serum concentrations using a chromogenic ecarin clotting assay has been previously reported by us with a very high correlation observed. Consecutive patients arriving at the University of California Davis Medical Center who received HD to expedite the removal of dabigatran were included.

Results: Four patients presenting with a major bleeding event requiring emergent drug reversal were included. Initial dabigatran serum concentrations ranged from 39 to 312 ng mL⁻¹. In the three cases with initial plasma concentrations above 200 ng mL⁻¹, a rebound in plasma dabigatran concentrations after stopping HD was observed. Prolonged HD was required to reach target goals for managing bleeding. Low dose aPCC (8–11 units kg⁻¹) immediately prior to dialysis catheter insertion was successful for access.

Conclusion: In this small case series, we report the successful use of a low dose of an aPCC (FEIBA) for catheter placement with subsequent HD and rebound in plasma dabigatran concentrations. For patients receiving chronic dabigatran, longer duration of HD, depending on the initial concentration, may be required for adequate drug removal.

Disclosure of Interest: None Declared.

PO359-WED

Effective use of low dose activated prothrombin complex concentrates in managing major GI bleeding on rivaroxaban

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Background: Limited information is available on managing major bleeding events during rivaroxaban therapy. Limited studies have explored various concentrated clotting factors, however their effects and the best dose in the setting of a major bleeding event is unclear.

Aims: To retrospectively evaluate our use of activated prothrombin complex concentrates (aPCC) in the management of major gastrointestinal bleeding (GIB) with concurrent rivaroxaban therapy. Low doses of aPCC may be effective in slowing a GIB and potentially reduce risks for subsequent thrombosis.

Methods: A consecutive series of patients receiving rivaroxaban admitted to the University of California, Davis Medical center requiring emergent hemostasis were retrospectively evaluated. Current institution guidelines for reversing the newer oral anticoagulants (NOAC) utilize a aPCC at low (~8 units kg⁻¹) for emergent reversal where some time is available to 25 units kg⁻¹ for exsanguinating bleeding, rounded to vial size. The bleeding, aPCC dose, rivaroxaban plasma concentrations using a chromogenic anti-factor Xa assay calibrated to rivaroxaban and outcomes were evaluated.

Results: Three patients with major lower (2) and upper (1) GIB were identified. Initial rivaroxaban plasma concentrations ranged 94 to 494 ng mL⁻¹, hemoglobin 5.7–7.8 with one patient going into cardiac arrest. Low dose of the aPCC FEIBA (9 to 14 Units kg⁻¹) was initially administered. Bleeding was noted to stabilize and stop shortly after administration and patients stabilized. Emergent esophagogastroduodenoscopy in the upper GI bleed indicated complete cessation of bleeding. All 3 patients were discharged without subsequent thrombosis noted.

Conclusion: In the setting of a GIB, low dose aPCC (9–14 units kg⁻¹) may be an effective adjunct to slowing or stopping bleeding for rivaroxaban.

This novel strategy may be effective for managing life threatening GIB with NOAC's if time permits as additional doses can be always given, while minimizing the risk for subsequent thrombosis.

Disclosure of Interest: None Declared.

PO360-WED

The cost-effectiveness of PD-APCC vs. RFVIIa in pediatric patients with haemophilia type A and inhibitors with mild to moderate joint bleeding in Colombia

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Background: The development of inhibitors is a serious and costly complication of haemophilia.

Aims: To compare the economic and clinical benefit of recombinant factor VIIa (rFVIIa) against plasma-derived activated prothrombin complex concentrate (pd-aPCC) in pediatric patients with haemophilia A and inhibitors with mild-to-moderate joint bleeding in Colombia and to support local decision making.

Methods: To determine the costs and effects of treating these patients in Colombia with rFVIIa and pd-aPCC, a decision tree was developed using TreeAge 2014. The perspective of the Colombian public healthcare provider was taken. Treur et al. 2009 literature review and public and official costs were used to populate the model. Inputs and model structure were validated and a maximum time horizon was set as 7 days by local Haemophilia experts (time all joint bleeds resolve if not by drug then spontaneously). Costs are presented in 2014 COL\$. To assess the impact of assumptions sensitivity and scenario analyses were performed (patient's weight, time horizon, efficacy, cost of treatments, of rebleeding and of hospitalization). Patients weight range was studied between 10 and 50 kg.

Results: At the 7th day, the cost per patient of treating mild-to-moderate joint bleeds was COL\$15,381,044 and COL\$18,759,671 with rFVIIa and pd-aPCC, respectively. At day 2, a time where most joint bleeds have resolved "rFVIIa is dominant, with cost per patient of COL\$12,951,702 compared to COL\$14,814,520 for pd-aPCC. rFVIIa provides an incremental positive bleeding resolution rate of 0.08. Patient's weight has an impact on drug costs and savings. At day 7, savings vary between COL\$1,604,920 and COL\$6,658,699. Treatment dosage and bleeding resolution rates are the main drivers of the model.

Conclusion: Against pd-aPCC, rFVIIa provides savings to the Colombian public healthcare provider due to earlier bleed resolution. Bleeding resolution rates and treatment dosing have impact on results.

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PO361-WED

Improving education and advocacy for young women with menorrhagia through use of innovative technology

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Background: Bleeding disorders (BD) occur in 20-50% of adolescents with menorrhagia (M), presenting challenges with BD information access, communication with medical providers, and compliance.

Aims: To use an innovative technological approach to enhance patient compliance with medications used to treat their M. To provide educational access to adolescents with BD.

Methods: Patients in the Young Women's Bleeding Disorder Clinic at Texas Children's Hospital were eligible. Participants filled out a quality of life survey and were given an iPod Touch® device (ITD), preloaded with the iPeriod application. Participants recorded in their ITD, information about their BD, menses, and medications. Data were collected to monitor compliance with prescribed treatment regimens. All ITD allowed WIFI access to allow teens to explore BD websites and knowledge was assessed.

Results: IRB approval was obtained from Baylor College of Medicine. Between 7/2010-12/2013, 46 consented, but 24 subjects completed within the time frame. Mean age: 14.1 ± 1.9 years. The population was diverse: 34% Caucasian, 4% Asian, 11% African American. Patients had a variety of BD resulting in M: 29% low von Willebrand (VW) factor activity, 54% VW disease, 12.5% platelet disorder, 4.2% factor deficiency. During the study, none became pregnant. At baseline, all subjects reported having difficulty participating in sports, low energy, and pain with their cycles. Subjects charted on menstrual flow (83.3%), cramps (100%), breakthrough bleeding (95.8%), mood (95.8%), and medication use (91.7%) for a mean of 9.3 ± 3.1 months. More than half used hormones to control M. No subjects stopped or missed medications. None required admission for M. Finally, all subjects reported accessing websites using their ITD to learn about their BD.

Conclusion: ITD is an excellent tool for adolescents with M and BD to allow self-monitoring, provider monitoring and improve educational access through engaging technology.

Disclosure of Interest: None Declared.

PO362-WED

Reduced hemostatic capacity after cardiopulmonary bypass

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Background: Disturbed coagulation and bleeding is a major clinical challenge after cardiopulmonary bypass (CPB).

Aims: To characterize the hemostatic changes in association with impairments of thrombin generation and fibrin formation after CPB.

Methods: Blood samples from 11 patients operated with CPB were collected at 4 time points: pre-operatively, and 10 min, 2 h and 24 h post-operatively. The following analyses were performed: Calibrated Automated Thrombogram (CAT), fibrinogen activity (Clauss) and

fibrinogen antigen, rotational thromboelastometry (ROTEM), fibrin network permeability, Scanning Electron Microscopy (SEM) and anti-FXa.

Results: Thrombin generation was significantly reduced at 10 min and 2 h after surgery, and normalized at 24 h. In parallel to changes in hematoric and hemoglobin, fibrinogen quantity (activity and antigen) declined at 10 min and 2 h after surgery, which was then significantly higher than the baseline after 24 h. The correlation between fibrinogen activity and antigen was strong ($r = 0.94$, $P < 0.001$). Fibrin network permeability (Ks) was increased 10 min and 2 h after surgery which then became normal at 24 h. SEM images support above findings in Ks. EXTEM-CT was significantly prolonged at 10 min after surgery returned then to baseline. FIBTEM-MCF was significantly reduced at 10 min and 2 h after surgery in agreement with fibrinogen activity/antigen measurements. Anti-FXa test showed a transient residual heparin effect.

Conclusion: There is a significant global reduction of hemostatic capacity early after CPB, including both impaired thrombin generation and fibrin formation capacity. Residual heparin effect and fibrinogen insufficiency due to plasma dilution during CPB may contribute. The impaired hemostatic capacity was normalized after 24 h indicating that the highest risk of bleeding is early after the operation.

Disclosure of Interest: None Declared.

PO363-WED

A fixed dose protocol for prothrombin complex concentrate does not lead to quicker and reliable intracranial hemorrhage reduction in vitamin K antagonist related intracranial hemorrhage

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Background: Millions of patients receive vitamin K antagonist (VKA) therapy worldwide. Annually 0.2-1% of all VKA users develops an intracranial hemorrhage (ICH). Prothrombin Complex Concentrate (PCC) is administered to restore the INR ≤ 1.5 in an attempt to limit hematoma growth. For non-intracranial bleeds, recent findings suggest that a lower fixed dose of 1000 IU FIX PCC might be non-inferior to the higher variable dose as recommended by the manufacturer in terms of hemostatic efficacy, as well as faster to administer. The optimal PCC dose for ICH is not clear. In order to optimize PCC dosing, our hospital recently introduced a fixed 1000 IU FIX PCC dosing protocol for ICH.

Aims: To retrospectively compare successful achievement of an INR ≤ 1.5 with a fixed dosing strategy vs. the variable dosing strategy of PCC in patients presenting with intracranial bleeding complications of VKA.

Methods: Two cohorts of patients were retrospectively compared by review of medical records. The fixed dose protocol cohort concerned the period December 2013 - August 2014 (cohort 1). Patients were compared to those who received a variable PCC dose in January 2013 - November 2013 (cohort 2). The medical ethics committee has approved the study.

Results: In cohort 1 a median dosage of 1000 IU PCC was given per patient ($n = 28$) compared to 1750 IU in cohort 2 ($n = 25$). In the intention-to-treat analysis, 68% achieved an INR ≤ 1.5 after an initial dose in the fixed dose cohort compared to 96% in the variable dose cohort ($P = 0.01$). An additional dose was given in 9 (32%) vs. 2 (8%) patients respectively ($P = 0.04$). The door-to-PCC-order time averaged 46 vs. 62 min ($P = 0.37$) and the door-to-needle time was 89 respectively 95 min ($P = 0.42$).

Conclusion: The fixed dose protocol necessitates additional PCC gifts more frequently to achieve a target $\text{INR} \leq 1.5$. Time to order and to needle were shorter but, in this small cohort, not significantly so. The effect on clinical outcome remains unknown.

Disclosure of Interest: None Declared.

PO364-WED

Risks and predictors of ischemic/bleeding complications in cardiac patients undergoing elective non-cardiac surgery – an analysis of the PRAGUE-14 study

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Background: The PRAGUE-14 is a prospective study that analyzed the impact of antithrombotic therapy interruption on outcomes in non-selected surgical patients with known cardiovascular disease.

Aims: To assess risks and predictors of ischemic/bleeding complications in consecutive patients with at least one cardiovascular disease undergoing elective major non-cardiac surgery.

Methods: Subgroup analysis of the PRAGUE-14 study was performed.

Results: Study population consisted of 1200 patients, 742 of them underwent elective surgery; age (median) 72.0, 66.2% males, CAD in 396, stroke in 70, atrial fibrillation in 209, venous thromboembolism (VTE) in 55, valvular heart disease in 108, artificial valve in 18, cardiomyopathy in 13. Preoperatively, 84.6% of patients were treated with antithrombotic therapy i.e. 439 aspirin, 31 DAPT, 192 oral anticoagulants. Prophylaxis of VTE in perioperative period was applied in 93.5%.

In-hospital ischemic complications occurred in 3% (vs 9.8% in acutely operated patients, $P < 0.001$), bleeding complications in 10.90% (vs 12.0%, n.s.), and both of them in 0.7% (vs. 4.2%, $P < 0.001$). Mortality of patients, who underwent planned surgery, was 2.2% (vs. 6.8% in acute surgery, $P < 0.001$).

Multivariate linear regression identified that pre-procedural anemia and history of (coronary or non-coronary) stent implantation were significantly related to ischemic complications. Periprocedural antithrombotic management has no impact on ischemic complications. Termination of warfarin less than 4 days before surgery was significantly related to the risk of bleeding.

Conclusion: Risk of perioperative ischemic complications is significantly lower in cardiac patients who are undergoing non-cardiac surgery electively (in comparison to acutely), and this risk is significantly related to a history of preprocedural anemia and history of stent implantation. For prevention from periprocedural bleeding, warfarin should be discontinued more than 4 days before surgery.

Disclosure of Interest: None Declared.

PO365-WED

The pharmacokinetics and immune response of new cationic dextran antidotes of heparin

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Background: We developed alternative, dextran-based, synthetic antidotes of unfractionated heparin (UFH) for replacement of protamine: 40kD dextran substituted with glycidyltrimethylammonium chloride groups at a ratio of 0.5 (Dex40-GTMAC2) or 0.65 (Dex40-GTMAC3) per glucose unit (Kaminski et al., J Med Chem, 2010). Dex40-GTMAC2 effectively reversed effects of UFH in a rat model of arterial thrombosis (Kalaska et al., Eur J Pharmacol, 2012).

Aims: The aim of the present study was to estimate distribution, elimination and immune response of the cationically modified dextran.

Methods: Animals were randomly divided into treatment groups: vehicle, UFH, Dex40-GTMAC2, Dex40-GTMAC3 and protamine. We confirmed efficacy of Dex40-GTMAC3 in the model of electrolytic inferior vena cava thrombosis induced in male BALB/c mice. We also evaluated immunogenic properties of polymers and compared them with protamine in a repeated-dose female BALB/c mice study. The elimination constant and half-life time was calculated in PK Solver Excel add-in program by measuring male Wistar rat plasma concentration of fluorescein-labelled Dex40-GTMAC2. The body distribution of fluorescein-labelled Dex-GTMAC2 or rhodamine-labelled Dex40-GTMAC3 was measured using In-vivo MS FX PRO system (Carestream Health INC., USA) in female NMRI-Foxn1nu/Foxn1nu mice. All the procedures involving animals and their care were approved by Local Ethical Committees.

Results: Cationic dextran antidotes of UFH are rapidly eliminated in the urine. They are present in the highly perfused organs such as liver, kidneys and lungs within 2 h after administration. Importantly, in contrast to protamine, dextran-based antidotes did not induce immune response.

Conclusion: Cationic dextran polymers are not only easy to synthesize and potent, but also safer than protamine, thus could be further evaluated in the clinical phase as novel antidotes of heparin.

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Disclosure of Interest: None Declared.

PO366-WED

Gender and bleeding in atrial fibrillation: a real-world prospective cohort study

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Background: In atrial fibrillation (AF), vitamin K antagonists (VKA) are frequently prescribed for stroke prevention, but increase the risk of bleeding. While female gender is an acknowledged risk factor for stroke, available literature remains ambiguous on gender as a risk factor for bleeding.

Aims: Our aim was to determine whether gender is associated with bleeding in patients on VKA treatment.

Methods: Patients from the outpatient anticoagulation clinic with AF using VKA were contacted in May 2011. All patients received a questionnaire regarding their medical status. Bleeding events were monitored until August 2012. The proportions of patients with either a clinically relevant or minor bleeding were compared between men and women. Clinically relevant bleeding comprised all bleeding events of critical organs, leading to death, contact for medical aid, or dose adjustment of the VKA.

Results: We included 2385 patients with a median age of 77 (IQR 69-83), of whom 56% were male. Women were significantly older, while no difference in HAS-BLED score was observed between sexes. The median follow-up of both groups was 1.3 years.

No significant difference between sexes was seen regarding clinically relevant bleeding (Table). Minor bleeding was observed significantly more often in females. After correction for age, gender was no longer significantly associated with minor bleeding.

	Male (N = 1330)	Female (N = 1055)	P-value
Patients with any bleeding (%)	225 (16.9)	205 (19.4)	0.11
Patients with clinically relevant bleeding (%)	92 (6.9)	76 (7.2)	0.79
Patients with minor bleeding (%)	152 (11.4)	153 (14.5)	0.03

Conclusion: In this real-world cohort of patients using a VKA for AF, gender was not independently associated with bleeding events. This supports current practice to not include gender in bleeding risk models.

Disclosure of Interest: None Declared.

PO367-WED

Low-dose 4-factor PCC for urgent warfarin reversal

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Background: Prospective studies evaluating prothrombin complex concentrates (PCC) for the reversal of warfarin-associated major hemorrhage (WAMH) excluded patients with a presenting INR < 2 and used a minimum dose of 25 units kg⁻¹. We developed a low dose 4-factor PCC protocol (LDPCC) for patients on warfarin with INR 1.6–1.9 and major hemorrhage or impending surgery.

Aims: To examine the utility LDPCC (15 u kg⁻¹) for the reversal of elevated INR and WAMH or impending surgery.

Methods: A weight-based dose of 15 u kg⁻¹ for an INR between 1.6 and 1.9 was adopted upon transition to 4-factor PCC at our institution in November 2013. This lower dose was also utilized regardless of INR in select patients requiring emergent procedures. All patients receiving LDPCC were prospectively entered into a registry managed by an anticoagulation pharmacist.

Results: 23 patients were treated with LDPCC for warfarin reversal. Indications for urgent reversal included: CNS hemorrhage (n = 15), impending surgery or procedure (n = 7), or bleeding from unknown source (n = 1). The median dose administered was 1090 (549-2565) units. Vitamin K was co-administered to 21 patients (91.3%) with 11, 8, and 2 patients receiving < 10 mg, 10 mg, and > 10 mg, respectively. The mean INR reduced from 2.51 ± 1.33 to 1.48 ± 0.45 on first repeat check (median 1 h, range 0.15-11.25 h). In 18 of 23 patients (78%) INR was ≤ 1.5 on first check, INR of 1.6 in 2 patients and 2 patients reversed from 3.2 to 2.6 and 7.9 to 2.9. In-hospital mortality for the entire cohort was 41.7%. Withdrawal of care occurred in

33.3%, with 16.7% of patients having withdrawal within 48 hours of admission. Thromboembolic events occurred in 2 patients (8.3%), the first an ischemic stroke 7 days post PCC, the second a DVT 19 days post PCC.

Conclusion: Utilization of a LDPCC (15 u kg⁻¹) together with vitamin K rapidly reversed the INR in most patients with WAMH or surgical indications for reversal. Given the thrombotic risks of receiving PCC, the minimum effective dose should be utilized.

Disclosure of Interest: None Declared.

PO368-WED

Development and validation of an assay for intra-surgical (or peri-operative) management of bleeding

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Background: Patients undergoing major surgical procedures are at high risk of excessive bleeding. HemoSonics LLC is developing the QuantraDx™ point-of-care (POC) system to guide transfusion selection during major surgery. The system provides time critical, quantitative information on hemostatic subsystems.

Aims: To develop a POC assay for the rapid identification of the presence of residual heparin, coagulation factor consumption, decreased platelet function and reduced fibrinogen concentration.

Methods: The assay was designed to measure four wells with different reagents (kaolin, thromboplastin, Heparinase I, polybrene, Reopro®) to generate clotting time and clot stiffness values that combined form indexes to guide patient treatment: residual heparin and consumption of factors with kaolin+/-Heparinase I, platelet function and fibrinogen levels with thromboplastin+/-Reopro. Optimal concentrations were established with titration experiments and tested with healthy donor blood and a novel control material to assess assay performance.

Results: Complete heparin reversal (6 IU mL⁻¹) was seen with 0.1 IU mL⁻¹ Heparinase I or 0.9 mg mL⁻¹ polybrene when activated with kaolin (0.1 mg mL⁻¹) or thromboplastin (1:50 dilution). Complete platelet inhibition was achieved with 0.7 mg mL⁻¹ Reopro with average clot stiffness at 290 Pa (3.3% CV). Control tests show average clotting times of 3–5 min with kaolin (n = 21) or thromboplastin (n = 28) with 4.8% and 9.4% CVs respectively. Average clot stiffness was 690 Pa for kaolin and 781 Pa for thromboplastin with CVs of 8.1% and 10% respectively. When testing the assay with whole blood, intra-cartridge average clotting time for kaolin activation was 3 min (5.3% CV), while average clot stiffness with thromboplastin+/-Reopro was 270 Pa (9% CV) and 2800 Pa (1.1% CV) respectively.

Conclusion: Data demonstrates the QuantraDx can provide time critical information to the surgeon regarding factor concentrations, platelet function, fibrinogen levels and the presence of heparin.

Disclosure of Interest: E. Ferrante Shareholder of: HemoSonics LLC, Employee of: HemoSonics LLC, K. Blasier Shareholder of: HemoSonics LLC, Employee of: HemoSonics LLC, T. Fischer Shareholder of: HemoSonics LLC, Employee of: HemoSonics LLC, F. Viola Shareholder of: HemoSonics LLC, Employee of: HemoSonics LLC.

PO369-WED

APTT screening test in the cohort of adult patients undergoing surgical procedures: insights for clinical daily practiceRadossi P¹, Zardo L², Salviato R¹, Chiappin S², Pierobon F¹, Floridia A¹, Piasterico G² and Tagariello G¹¹Transfusion and Haematology; ²Laboratory, Castelfranco Veneto Hospital, Castelfranco Veneto, Italy**Background:** In Italy coagulation screening prior surgical procedures is routinely performed with the scope to guide the operative care.**Aims:** Evidence from medical and surgical literature suggests that elongated aPTT, considered independently, is not adequate to predict patients' bleeding risk.**Methods:** We have evaluated aPTT in the cohort of patients undergoing elective surgery from 1st January to 31st December 2014 a total of 8071 patients were screened.**Results:** In 7606 (94.2%) aPTT ratio was within the normal range while 465 (5.8%) was abnormal. 223 were between 1.2 and 1.3 ratio and we considered these setting not suitable of further investigations. In 242 patients it was higher than 1.3 and in this subgroup the vast majority (201pts, 83%) were on oral anticoagulation therapy (OAT) explaining the abnormality because of the involvement of the vitamin K dependent factor IX, 3 were not confirmed when repeated and 2 were already known as mild haemophilia with FVIII 9% and 18% respectively. 4 patients were affected by chronic liver diseases. 11 cases were tested for intrinsic pathway and 7 cases were identified as lupus anti coagulant, 3 mild FXII deficiency (53%, 51% and 38% vs normal value > 70%) one mild FXI deficiency (50% vs normal value > 70%) 21 patients underwent to operations without any other clarifications of the defect but surgery was performed safely.**Conclusion:** in the preoperative setting our results seem to confirm the low prevalence of coagulation defects in the general population. Screening clotting tests and aPTT ratio elongation seem in relationship mainly with OAT while the rest is represented by mild coagulation defects without any predictive meaning about the bleeding risk. On the contrary accurate anamnestic personal history, including medications (mainly OAT) seems more useful to predict the risk of bleeding and coagulation tests abnormality. According to our results we think that preoperative lab tests should not be performed routinely to guide the perioperative care.**Disclosure of Interest:** None Declared.

PO370-WED

Intraventricular fibrinolysis restores glymphatic circulation after subarachnoid hemorrhage

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*Serine Protease and Pathophysiology of the Neurovascular Unit, INSERM U919, Caen, France***Background:** Whereas we thought that the brain was devoid of lymphatic circulation, a recent study has demonstrated that its role is played by a para-vascular circulation of cerebrospinal fluid (CSF) inside the brain parenchyma: the glymphatic system. This glymphatic system is especially important for brain waste clearance and thus, neuronal survival. We hypothesized that following subarachnoid hemorrhage, the release of blood in the CSF may impair glymphatic functioning and worsen neurological outcome.**Aims:** (i) To investigate whether the function of the glymphatic system was impaired following subarachnoid hemorrhage and (ii) to test therapeutic strategies to improve glymphatic perfusion.**Methods:** Subarachnoid hemorrhage induced by injection of blood in the pre-chiasmatic cistern of mice.**Results:** We first developed a non-invasive method to measure the glymphatic perfusion using magnetic resonance imaging (MRI) in mice. Using this new technique, we demonstrated that the glymphatic perfusion was completely blocked after subarachnoid hemorrhage. Immunohistological studies provided evidence that the deposition of fibrin in the perivascular spaces was responsible for this glymphatic impairment. We therefore investigated whether intraventricular fibrinolysis (i.e. injection of a fibrinolytic in the CSF) was able to improve glymphatic perfusion *via* fibrin degradation. Interestingly, a unique injection of tPA in the right ventricle of hemorrhagic mice significantly improved glymphatic perfusion.**Conclusion:** Intraventricular fibrinolysis improves glymphatic perfusion after subarachnoid hemorrhage. This therapeutic strategy may be of clinical value for patients presenting ruptured intracranial aneurysms for instance. Whether other neurological conditions are associated with reduced glymphatic perfusion due to fibrin deposition remains to be investigated.**Disclosure of Interest:** None Declared.

PO371-WED

Evaluating the inter-observer reliability of potential predictor variables to develop a more accurate prediction tool for bleeding for patients on oral anticoagulantFallah P¹, Wells PS^{1,2,3} and Forgie MA^{1,2,3}¹Ottawa Hospital Research Institute; ²Department of Medicine;³University of Ottawa, Ottawa, Canada**Background:** Bleeding is the major complication of oral anticoagulant therapy (OAT). The risk of major bleeding is an important consideration in determining the optimal duration of OAT. In order to optimize long-term treatment decisions for doctors and patients with venous thromboembolism (VTE), a valid predictive index to determine bleeding risks while taking long-term anticoagulation is essential. In order to derive a prediction tool it is necessary to ensure the included variables are reproducible when chosen by different observers.**Aims:** We sought to assess the inter-observer reliability of potential predictor variables to develop a more accurate prediction tool for bleeding for patients on extended OAT (more than 3 months of OAT) for VTE.**Methods:** Bleeding Risk is a multicentre, multinational prospective cohort study of patients on extended OAT for VTE. Patients were enrolled in the study after at least 3 months of OAT. Patients were followed every 6 months for bleeding events. All major bleeding events during long term OAT were captured and adjudicated. For 70 patients duplicate independent examinations were conducted to determine the inter-observer reliability of 15 predictor variables that could be subject to interpretation or recall bias. The inter-observer reliability of each predictor variable was determined by calculating a two-rater unweighted Kappa statistic.**Results:** We calculated the inter-observer reliability of 15 individual potential bleeding predictor variables for 70 study participants in Ottawa. The Kappa scores were higher than 0.8 for current smoker, treatment for hypertension, atrial fibrillation, diabetes mellitus, post-thrombotic syndrome, prior CVA, prior gastrointestinal bleed and 5 current medications and were more than 0.6 for prior myocardial infarction and 2 current medication variables.**Conclusion:** The 15 potential variables had good and excellent reliability and can be used to develop the bleeding prediction tool for patients on extended OAT for VTE.**Disclosure of Interest:** None Declared.

PO372-WED

Effects of hemostatic gauze with self-propelling thrombin particles on survival and blood loss in a swine model of lethal arterial hemorrhageJohn AS¹, Baylis JR², Wang X¹, Lim EB¹, Chien D¹, Stern SA¹, Kastrup CJ² and White NJ¹¹Medicine, University of Washington, Seattle, USA; ²Michael Smith Laboratories, University of British Columbia, Vancouver, Canada

Background: Severe bleeding from large arterial wounds is a leading cause of death after trauma. Hemostatic gauzes containing hemostatic activators are currently used to pack and compress wounds but are less effective in anatomical locations where compression cannot be performed. Gauze impregnated with self-propelling hemostatic particles that disperse hemostatic activators throughout the wound may be useful in these situations.

Aims: Compare hemostatic gauze to gauze with self-propelling hemostatic particles for wound packing in a swine model of lethal noncompressible femoral artery hemorrhage.

Methods: A self-propelling hemostatic bandage was created by binding thrombin to calcium carbonate microparticles and coating these particles on cotton bandage in the presence of solid protonated tranexamic acid (TXA). Contact with blood causes brisk release of CO₂ and propulsion of microparticles against flowing blood to deliver the thrombin and induce hemostasis. Non-protonated TXA was substituted to form a non-propelling control. Bleeding was induced in 22 immature female swine using a 5 mm femoral arteriotomy. Pigs were randomized into 1 of 3 packing interventions without wound compression: kaolin hemostatic gauze (*Combat Gauze*TM) (KG), propelled thrombin gauze (PTG), or non-propelled thrombin gauze (NPTG). All animals then received intravenous fluids to restore blood pressure for up to 3 h. Survival was compared using Kaplan-Meier log rank, and blood loss was compared using ANOVA.

Results: Survival was improved with PTG (3-hr survival: 7/7, 100%) compared to KG (2/7, 28.6%, $P = 0.01$), and compared to NPTG (2/8, 25.0%, $P = 0.01$). Total and post-intervention external blood loss were similar in all groups ($P > 0.05$).

Conclusion: Propelled thrombin gauze improved survival in this lethal bleeding model. Self-propelling hemostats may enhance hemostasis in major wounds.

Disclosure of Interest: None Declared.

PO373-WED

Clinical management and outcome of major bleeding in patients on treatment with Vitamin K antagonists: results from the climbing studyFranco L¹, Becattini C¹, Masotti L², Nitti C³, Cattinelli S⁴, Cappelli R⁵, Manina G⁶, Sbrojavacca R⁷, Pomerio F⁸ and Agnelli G¹

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Background: The optimal treatment of vitamin K antagonists (VKAs)-induced major bleeding (MB) and the effect of management on clinical outcome remain unclear.

Aims: The aim of the study was to assess the actual management and the determinants of outcome of VKA-associated bleeding.

Methods: Patients hospitalized for MB while on treatment with VKAs were included in a prospective, cohort study. MB was defined according to the ISTH criteria. The primary study outcome was death occurring during the hospital stay. The secondary outcome of the study was major thromboembolic complications related to treatment or anticoagulation withdrawal.

Results: 450 patients were included in this study, of which 243 with intracranial hemorrhage (ICH).

Prothrombin complex concentrates (PCCs) were used in 50% and in 23% of patients with ICH or non-intracranial MB, respectively ($P < 0.001$); fresh frozen plasma (FFP) was used in 7% and in 20% of patients with ICH or non-intracranial MB ($P < 0.001$).

Death occurred in 89 patients during the hospital stay (20%), 62 patients with ICH and 27 patients with non-intracranial MB. Age, ICH, low GCS score and shock were independent predictors of in-hospital death. INR at admission, trauma and HASBLED of 3 or higher (or any of its component) were not associated with adverse outcome.

The incidence of arterial thromboembolic complications was 7% in patients who received PCCs and 4.3% in those who did not receive PCCs.

Conclusion: Among patients hospitalized for MB while on VKAs, the risk for death is substantial. Age, ICH, low GCS score and shock at presentation were associated with in-hospital death.

Disclosure of Interest: None Declared.

PO374-WED

Comparative studies on a branded recombinant factor VIIa and its generic version to demonstrate equivalenceSadeghi N¹, O'Malley P¹, Iacobelli M¹, Vaziri B², Syed D¹, Kahn D¹, Hoppensteadt D¹, Guler N¹ and Fareed J¹¹Pathology, Loyola University Medical Center, Maywood, USA;²Aryogen, Tehran, Iran, Republic of Islamic

Background: Commercially available recombinant factor VIIa (Novoseven) is widely used in the management of hemophilia patients with inhibitors. Recently several generic versions of recombinant VIIa (rFVIIa) have become available. The generic versions of rFVIIa are claimed to be biosimilar to the Novoseven.

Aims: The purpose of this study is to compare the US and European Novoseven products with a generic version of rFVIIa namely, Aryoseven.

Methods: Four commercially available lots of Novoseven were obtained from the US and European sources. Four different batches of Aryoseven were obtained from Aryogen (Tehran, Iran). All individual rFVIIa preparations were diluted to obtain working concentrations of 100, 10, 1 and 0.1 ug mL⁻¹. Protein content (Lowry's method), molecular profile using surface enhanced laser desorption ionization (SELDI), gel electrophoretic profile (GEP), factor VII related antigen level (F:VII:Ag), correction studies in factor VII depleted plasma and tissue factor mediated thrombin generation (TG) studies were carried out.

Results: The protein content and SELDI mass spectrophotometric profile of all 4 rFVIIa preparations were comparable. There was no differences in the Novoseven obtained from the US and European sources. The GEP of the two groups of agents showed a comparable profile with distinct peaks at 50 and 25 kDa. The FVII related antigen levels were also comparable in the Novoseven and Aryoseven preparations. Supplementation of both the Novoseven and Aryoseven preparations at 10–100 ng mL⁻¹ resulted in a comparable correction of the factor VII deficient plasma as measured by PT(INR). All batches of the Novoseven and Aryoseven produced comparable thrombin generation in PCC based systems.

Conclusion: These results indicate that the US and European Novosevens are comparable. Four batches of Novoseven and 4 individual

clinical batches of Aryoseven were found to be bioequivalent. Thus the generic Aryoseven is biosimilar to branded Novoseven and warrant clinical validation studies.

Disclosure of Interest: N. Sadeghi: None Declared, P. O'Malley: None Declared, M. Iacobelli: None Declared, B. Vaziri Employee of: Aryogen, D. Syed: None Declared, D. Kahn: None Declared, D. Hoppensteadt: None Declared, N. Guler: None Declared, J. Fareed: None Declared.

PO375-WED

Self-propelling particles that transport thrombin to manage hemorrhage

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Background: Delivering coagulants to the site of bleeding is challenging due to the outward flow of blood and uncontrolled bleeding is a major cause of surgical complication. Self-propelling particles have been proposed as a strategy for transporting agents *in vivo* and are a promising avenue for designing new therapeutics aimed at managing hemorrhage.

Aims: We aim to load thrombin onto self-propelling particles consisting of CaCO₃ and tranexamic acid (TXA) and to test this formulation's ability to manage hemorrhage using two mouse models.

Methods: Self-propelling CaCO₃ microparticles were synthesized via simple precipitation and solid-solid mixing with organic acids such as TXA. Thrombin was adsorbed onto microparticles by non-specific adsorption in aqueous suspension. Thrombin-loaded, self-propelling CaCO₃ microparticles (propelled thrombin) were tested for their ability to clot blood *in vitro*, in a mouse tail clip model of amputation and in a mouse liver puncture model of surgical bleeding.

Results: Propelled thrombin clotted flowing plasma *in vitro* significantly faster than non-propelling controls. Propelled thrombin significantly reduced bleeding time and blood loss, respectively, in a mouse tail clip model of hemorrhage and in a mouse liver puncture model of surgical bleeding. Histological analysis showed propulsion increased penetration and localization of the particles to sites of injury in both mouse tails and mouse livers.

Conclusion: We have successfully loaded simple, self-propelling particles consisting of CaCO₃ and TXA with thrombin to create an effective hemostatic agent. This agent reduced bleeding in two mouse models of hemorrhage by transporting thrombin and burrowing deep into wounds. This material may have promising applications in managing more severe forms of hemorrhage, or for delivering other classes of therapeutics such as fibrinolytics.

Disclosure of Interest: None Declared

Megakaryocytes and thrombopoiesis

PO376-WED

A microfluidic chamber for elongating megakaryocytes and producing functional platelets

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Background: Platelets are formed by fragmentation of megakaryocytes (MK). We previously found that exposing MK to shear and von Willebrand Factor (VWF) accelerates platelet production. To mimic MK passage through bone marrow sinusoids, bioreactors have been engineered, consisting of a porous membrane separating a MK reservoir from a flow channel where MK fragments are subjected to shear. However these bioreactors did not allow for efficient and rapid platelet production.

Aims: To build a microfluidic system where MK are directly injected in the chamber at a high shear allowing for their capture, subsequent elongation, fragmentation into (pro)platelets, and rapid collection of newly formed platelets for their characterization.

Methods: The chamber is made of a rectangular microchannel with an array of VWF-coated micropillars used as anchors for MK. 2 large inlet and outlet cavities are connected to 16 parallel identical channels, with a 13.2 cm length of each textured channel. Mature MK obtained by cord blood CD34⁺ cell culture are perfused through the chamber and recirculate for 2 h at high shear. Platelets are then collected, separated from intact MKs via an albumin gradient and characterized *in vitro*.

Results: MK are anchored onto VWF-coated micropillars with a high efficiency. Once trapped on a pillar they are subjected to shear, producing elongations that break into proplatelets and platelets. Time lapse observations reveal that the fragments released by a single anchored MK can represent 350 platelets in terms of volume. Platelets express physiological levels of GPIb and α IIB β 3. Upon SFLLRN or thrombin addition to the suspension, they undergo activation as shown by their cytoskeletal reorganization, PAC1 binding, and they form large aggregates in contrast to control samples (not passing through the chip) that fail to do so.

Conclusion: This chamber allows high MK throughput and rapid formation of platelets with preserved functions, opening up the possibility of platelet production for therapeutic use.

Disclosure of Interest: A. Blin Employee of: PlatOD, A. Le Goff: None Declared, S. Poirault-Chassac Consultant for: PlatOD, G. Sicot Employee of: PlatOD, B. Teste: None Declared, A. Magniez Employee of: PlatOD, M. Reyssat: None Declared, D. Baruch Shareholder of: PlatOD.

PO377-WED

Novel roles of the dual-specificity phosphatases 1 and 6 in megakaryopoiesis and proplatelet formation

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Background: Extracellular signal-regulated kinases-1/2 (ERK1/2) are essential for transmitting signals across the nuclear membrane and regulating gene expression during megakaryopoiesis. However, it remains unclear how these signals are inhibited during proplatelet formation. Dual-specificity phosphatases (DUSPs) comprise a family of serine/threonine and tyrosine protein phosphatases that negatively regulate

mitogen-activated protein kinases (MAPKs). DUSPs have been shown to be critical regulators of the immune response however; their roles in megakaryopoiesis have yet to be explored.

Aims: We investigated the expression and cellular functions of DUSP1 and DUSP6 during megakaryocyte (MK) development and proplatelet formation.

Methods: Mouse primary bone marrow-derived MKs were treated with the DUSP1/6 inhibitor BCI. MK function and signaling were analysed using standard protocols.

Results: We show that DUSP1/6 expression increases during MK development, peaking in mature MKs. ERK1/2 phosphorylation was used as an indicator of activity. Thrombopoietin (TPO)-induced ERK1/2 activation is dramatically decreased at late stages of megakaryopoiesis, inversely correlated with DUSP1/6 expression. Peak ERK1/2 phosphorylation was detected in immature MKs 10 min post TPO stimulation, which contained the lowest levels of DUSP1/6. However, at late stages of megakaryopoiesis, TPO-induced ERK1/2 phosphorylation was decreased. Moreover, basal and TPO-induced ERK1/2 phosphorylation was increased in the presence of BCI. Interestingly, MK ploidy and the percentage of proplatelet-forming MKs was increased in the presence of BCI in a dose dependent manner, suggesting that DUSP1/6 inhibit platelet formation.

Conclusion: Findings from this study suggest that DUSP1/6 work in conjunction to inhibit Erk1/2 activity during MK development and platelet production. This study provides important insights into the molecular mechanisms regulating MK and platelet production.

Disclosure of Interest: None Declared.

PO378-WED

Functionally active tissue factor is expressed during megakaryocyte maturation and is transferred to a subset of platelets

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Background: Tissue factor (TF) has been shown to be expressed also by platelets. Although both megakaryocytes (MK) and platelets express TF mRNA and platelets are able to make *de novo* protein synthesis, the main mechanism claimed to be responsible for the presence of TF within platelets is through the uptake of TF positive microparticles.

Aims: To assess whether human MK synthesize TF and transfer it to platelets.

Methods: In order to avoid the cross-talk with circulating microparticles, we took advantage from an *in vitro* cultured megakaryoblastic cell line (Meg-01) able to differentiate into megakaryocytes releasing platelet-like particles (Meg-platelets) upon treatment with valproic acid (2 mM). TF expression and activity were evaluated by different approaches in human megakaryoblasts, megakaryocytes and platelets. To confirm that TF expression documented in Meg-01 was not a feature of the transformed cell line we analysed TF expression in human CD34^{pos}-derived MK (CD34^{pos}-MK) and in their released platelets (CD34^{pos}-platelets).

Results: Real-time PCR experiments showed a 3 fold higher expression of TF mRNA in Meg-megakaryocytes compared to Meg-megakaryoblasts. Immunoassays showed that also TF protein was significantly more abundant in Meg-megakaryocytes than in Meg-megakaryoblasts (90 ± 15 and 50 ± 10 pg mg⁻¹ cell protein, respectively) and this was

paralleled by a significantly higher thrombin generation capacity in Meg-megakaryocytes compared to Meg-megakaryoblasts. Flow cytometry analysis showed that TF was present in the cytoplasm of 40% of Meg-platelets where it was functionally active and blocked by an anti-TF Ab. Both TF pre-mRNA and mRNA were detected in Meg-platelets and all these data were confirmed in CD34^{pos}-MK and CD34^{pos}-platelets.

Conclusion: Functionally active TF is expressed in human megakaryoblasts, megakaryocytes and in their shedded platelets. These data indicate that platelets express TF independently of the interaction with other cells.

Disclosure of Interest: None Declared.

PO379-WED

Functional analysis of NBEAL2 in human megakaryocytes via colocalization with subcellular compartments

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Background: Mutational loss of NBEAL2 function causes Gray Platelet Syndrome (GPS), where platelet α -granule membranes are present, but granule cargo is absent. NBEAL2 is a BEACH-domain containing protein with no known function.

Aims: To explore the role of NBEAL2 in α -granule biogenesis by analyzing its subcellular localization in human megakaryocytes.

Methods: CD34⁺ cells affinity-purified from peripherally mobilized blood were cultured with thrombopoietin for 12 days to stimulate megakaryocyte differentiation. To preserve structure integrity cells were seeded onto a matrix that simulates the marrow environments for the last 4 days prior to fixation. Megakaryocytes were immunostained with anti-NBEAL2 antibody in combination with a range of cellular compartment markers, and imaged via laser fluorescence confocal microscopy. Images were analyzed for signal colocalization, which was quantified via the corrected Pearson correlation coefficient.

Results: NBEAL2 in human megakaryocytes showed a primarily cytoplasmic distribution pattern, with significant colocalization with the α -granule markers P-selectin (0.431 ± 0.050) and VAMP8 (0.600 ± 0.083), which also colocalize with each other. No significant association was observed between NBEAL2 and the nucleus, endoplasmic reticulum, Golgi apparatus, early endosomes, late endosomes or dense-granules. This suggests that NBEAL2 may be involved in the late stages of α -granule assembly and/or cargo loading.

Conclusion: In human megakaryocytes NBEAL2 colocalizes with the α -granule markers P-selectin and VAMP8. This protein is unlikely to be involved in early events of α -granule formation, but NBEAL2 may play a role in later events such as the loading of cargo proteins into α -granules.

Disclosure of Interest: None Declared.

PO380-WED

CCL5 released from platelets drives increased megakaryocyte maturation and proplatelet formation

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Background: In times of physiological stress (i.e. myocardial infarction, infection, inflammation, malignancy), platelet count can elevate

transiently. What initiates this reactive thrombocytosis is poorly understood. Platelets store inflammatory cytokines that are released upon activation, including abundant amounts of chemokine ligand 5 (CCL5). We hypothesize that CCL5 could self-regulate platelet production through a positive feedback mechanism targeting megakaryocytes (MKs).

Aims: Establish that platelet-derived CCL5 drives auto-regulation of transient changes in platelet production.

Methods: Primary murine fetal liver-derived MKs were treated with CCL5 or platelet releasate and their number and ploidy were quantified by flow cytometry and proplatelet formation quantified in ImageJ. CCL5 and its receptor CCR5 were characterized in MKs and platelets using western blot, flow cytometry and immunofluorescence. CCL5 in platelets was quantified with ELISA.

Results: CCL5 is present in both MKs and platelets but CCR5 expression is restricted to MKs. MKs cultured with CCL5 increased proplatelet production by 50%. MKs treated with CCL5 had significantly higher ploidy; 65% fewer MKs were 2N while 76% more were 16N. Pretreating the MK cultures with Maraviroc (CCR5 antagonist) prior to exposure to CCL5 reversed the augmented proplatelet formation and ploidy, suggesting the effect is mediated through CCR5. Upon activation with TRAP, platelets release over 1.8 pg mL^{-1} of CCL5 and this releasate significantly increased proplatelet production by MKs 47%. Importantly, Maraviroc diminished 95% of the effect of platelet releasate on proplatelet production, suggesting the CCL5 derived from platelets is sufficient to drive increased platelet production through MK CCR5.

Conclusion: CCL5 increases MK ploidy and subsequent proplatelet formation in a CCR5-dependent manner. We propose that CCL5 may act in addition to TPO to increase platelet counts during physiological stress.

Disclosure of Interest: None Declared.

PO381-WED

Anti-gpiib antibody inhibits platelet production from megakaryocytes, but not megakaryocyte maturation, analyzed in a murine model of immune thrombocytopenia

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Background: Immune thrombocytopenic purpura (ITP) is an autoimmune hemorrhagic disorder. However, it is unknown whether platelet (plt) production is affected by anti-plt antibody (Ab).

Aims: In this study by using a murine model of ITP, we investigated the influence of anti-plt Ab on megakaryopoiesis.

Methods: Proplatelet formation (PPF) and CFU-MK were analyzed by culturing MKs or lineage-/c-kit+ (L-/K+) cells in the presence of anti-GPIIb (Pm1) or anti-GPIb α Ab. To analyze the effect of anti-plt Ab in a murine model of ITP, Pm1 was repetitively administered intraperitoneally, and MK counts and PPF were analyzed.

Results: When MKs were cultured in the presence of various concentrations Pm1, PPF was dose-dependently inhibited. Although number of plt buds in proplatelet-forming MKs was reduced in the presence of Pm1, size of plt buds was increased. Addition of anti-GPIb α Ab inhibited PPF. Contrary to these results, CFU-MK and MK ploidy were not affected by adding Pm1 when L-K+ cells were cultured with TPO. We analyzed the effect of Pm1 in a murine model of ITP. Observation of *ex vivo* culture of MKs revealed that PPF was abrogated in MKs obtained from Pm1-injected thrombocytopenic mice. Moreover, plt size in peripheral blood was increased than that in control mice. In contrast, number of MKs was rather increased in these thrombocyto-

penic mice. These results suggest that plt production is abrogated in Pm1-injected mice, which corresponds well with the result of *in vitro* culture. Finally, to exclude the possibility that apoptosis in MKs is responsible for thrombocytopenia in ITP, which was argued in a previous report, we assayed whether caspase-3 activation (cas3-a) in MKs is induced by Pm1. However, cas3-a was not observed in primary MKs with Pm1 or in MKs obtained from Pm1-injected mice.

Conclusion: We conclude that anti-plt Ab impairs plt production from MKs without affecting MK maturation, and this mechanism as well as disruption of circulating plts underlies thrombocytopenia in ITP.

Disclosure of Interest: None Declared.

PO382-WED

Plant food cyanidin-3-O-B-glucoside promoted megakaryopoiesis by regulation of the apoptotic pathway in dami and Meg-01 cell lines *in vitro*

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Background: The number of circulating platelets is associated with cardiovascular events. Anthocyanins are major phytochemicals abundant in plant food and have been shown to play a protective role against CVDs, and our previous studies has proved that anthocyanins can promote apoptosis of activated platelets *in vitro* and *in vivo* (unpublished data) without platelets number decrease and bleeding problem. Megakaryocytes (MK) are highly specialized precursor cells to produce and release platelets into the circulation, however, the direct effects and mechanisms of anthocyanins on megakaryocytic proliferation and differentiation have not been studied.

Aims: To investigate the effects of anthocyanins on megakaryopoiesis *in vitro*.

Methods: Cyaniding-3-gulucoside (Cy-3-g), the predominantly bioactive compounds of anthocyanin preparations, was obtained from Polyphenol AS Company. Human megakaryocytic cell lines, Dami and Meg-01 were incubated with different concentrations (0.05, 0.5, 5 and $50 \mu\text{mol L}^{-1}$) of Cy-3-g or PBS buffer as a control. Cell proliferation was assayed by colony-forming unit-megakaryocyte (CFU-MK). Flow cytometry and Giemsa staining analysis were used to confirm the stimulating effect of Cy-3-g on CD41⁺ and polyploidization of nuclear. Extrinsic and intrinsic apoptosis pathways of Mk also were evaluated by western blotting.

Results: The data indicated that Cy-3-g at 50 and $100 \mu\text{mol L}^{-1}$ significantly stimulated CFU-MK on both MK cell lines *in vitro*. The promote effects of Cy-3-g at 50 and $100 \mu\text{mol L}^{-1}$ on the percentage of CD41⁺ in megakaryocyte and polyploidization of nuclear were also observed. Cy-3-g significantly inhibited expression of BCL-xL, increased the levels of Bax, Bak, caspase-9, 8 and 3 in a dose dependent manner, however there was no significant difference in Fas protein expression.

Conclusion: Our data clearly demonstrated for the first time that Cy-3-g directly accelerated proliferation and differentiation of human megakaryocytic cell lines through promoting intrinsic apoptosis pathway.

Disclosure of Interest: None Declared.

PO383-WED

Megakaryocytes and platelets express nicotinic acetylcholine receptors but nicotine does not affect megakaryopoiesis or platelet function

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Background: In our previous investigations we have shown that platelets and their precursors express nicotinic $\alpha 7$ acetylcholine receptors (nAChR $\alpha 7$) that are involved in platelet function and *in vitro* differentiation of the megakaryoblastic cell line MEG-01.

Aims: In this study, we were interested in the expression analysis of additional nAChR and the effects of nicotine in an *ex vivo* model using megakaryocytic cells differentiated from cord blood derived CD34⁺ cells (CBMK) and an *in vivo* model using blood samples from smokers.

Methods: CBMK were differentiated with thrombopoietin (TPO) for up to 17 days. Quantitative real-time PCR (QRT-PCR), Western blot analysis and flow cytometry were used to investigate nAChR expression (nAChR $\alpha 7$, nAChR $\alpha 4$, nAChR $\beta 2$) and nicotine effects. In blood samples of 15 nonsmokers and 16 smokers platelet parameter (count, MPV, PDW) were determined as indicators for changes of *in vivo* megakaryopoiesis. Platelet function was determined by the use of whole blood aggregometry and flow cytometry. The functional role of nAChR was evaluated using specific antagonists in aggregometry.

Results: *CHRNA7*, *CHRNA4* and *CHRN2* gene transcripts and the corresponding proteins could be identified in CBMK during all stages of differentiation. Platelets contain nAChR $\alpha 7$ and nAChR $\beta 2$ but not nAChR $\alpha 4$. Nicotine had no effect on TPO-induced differentiation of CBMK. There was no significant difference in all platelet parameter of smokers compared to nonsmokers. In line with this, cholinergic gene transcripts as well as the encoded proteins were equally expressed in both study groups.

Conclusion: Despite our observation of nAChR expression in megakaryopoiesis and platelets, we were not able to detect effects of nicotine in our *ex vivo* and *in vivo* models. Thus, the functional role of the nAChR in these cells remains open.

Disclosure of Interest: None Declared.

PO384-WED

Novel regulators of megakaryopoiesis by ribosome-protected RNA sequencing

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Background: Megakaryopoiesis is tightly orchestrated by changes in gene expression. It is known that microarray or RNA-seq provide a poor proxy for protein abundance ($R \approx 0.4$), while protein translation rates obtained by sequencing ribosome-protected RNA footprints (Ribo-seq) correlate at $R \approx 0.8$ with protein levels. Therefore, Ribo-seq represents a powerful tool to study gene expression during megakaryopoiesis.

Aims: To identify novel regulators in megakaryopoiesis by Ribo-seq.

Methods: MKs were obtained by culture of cord blood CD34⁺ cells. Translation was stalled by cycloheximide before sorting immature (CD41⁺/42b⁻) and mature (CD41⁺/42b⁺) MKs. Ribosomal foot-

prints from 3 biological replicates each were sequenced on the Illumina HiSeq and analysed using MMDIFF. Genes with a posterior probability > 0.5 were considered differentially translated (DT).

Results: Identification of known MK regulators confirmed the robustness of the method; i.e. TAL1 and pleckstrin translation increased in CD42b⁺ MKs and translation of CD34 and MYB arrested. Overall 299 protein-coding genes were identified as DT between CD42b⁻ and CD42b⁺ MKs. Down-regulated genes were enriched in glycoproteins/plasma membrane proteins and up-regulated ones showed enrichment for proteins with SH3 domains and roles in cytoskeletal organisation and apoptosis. Of the DT genes, 20 occur in a database of ~1200 mouse knock-outs with megakaryocyte and platelet related phenotypes, including RAC1. On inspection of 714 genome sequenced patients with platelet disorders from the BRIDGE cohort, a missense variant was identified in RAC1 for 3 patients, which is absent from ~80,000 controls, suggesting its role in MK maturation.

Conclusion: With a high level of accuracy, Ribo-seq determines the extent of translation and the repertoire of isoforms, with evidence for new reading frames and extensive post-transcriptional regulation. Knowledge of the DT genes allows a better delineation of the molecular controls at play during the final stage of MK maturation.

Disclosure of Interest: None Declared.

PO385-WED

Gene expression profile in megakaryocytes from type 2 diabetic mice emphasizes increase in stefina expression levels

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Background: Until now, knowledge about platelet alterations in type 2 diabetes mellitus (T2DM) was obtained based on well-established platelet properties.

Aims: Our goal was to identify new deregulated pathway in platelets specific to T2DM using the advantages of an unbiased microarray approach. We studied the modifications in gene expression occurring in megakaryocytes (MKs), during T2DM.

Methods: Using the Agilent platform array with the Whole mouse Genome chip (8 × 60k), we analyzed the gene expression of bone marrow-isolated MKs from diabetic (db/db) and wild-type (Wt) mice. Gene's differential expression was then confirmed by qPCR and western blot.

Results: Eighty-three genes were differentially expressed in diabetic mice (fold change (FC) > 1.5 and $P < 0.05$) as compared to Wt. Functional GO and KEGG annotations revealed that up-regulated genes belong to calcium (30%) and G protein-coupled receptor (31%) signaling pathways as well as complement and coagulation cascade (14%). *Stefin A* present the highest FC in db/db MKs (FC=9.7, $P < 0.001$). This up-regulation was confirmed by qPCR (FC=13, $P = 0.0047$) and western blot at the protein level. *Stefin A*, mouse homolog for the human *Cystatin A* (*CSTA*), encodes a cysteine proteinase inhibitor involved in cell adhesion and proliferation. *CSTA* also acts as anti-apoptotic and anti-angiogenic molecule. *CSTA* mRNA and protein levels increase during differentiation of human CD34⁺-derived MKs. *CSTA* is present in human platelets and released upon agonist-induced activation.

Conclusion: We highlighted that diabetic status modify the gene expression patterns in mice MKs strongly increasing *Stefin A* levels. We show for the first time that *CSTA* is expressed in both human MKs and platelets. We will now place efforts in understanding *CSTA* role in MKs and platelet functions and in T2DM-associated vascular complications.

Disclosure of Interest: None Declared.

PO386-WED

Identification of megakaryocytic progenitor cells among subcutaneous pre-adipocytes

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Background: Megakaryocytes (MKs) and platelets are generated from pre-adipocytes (PAs), and much attention has been paid to clarify the mechanism. PAs are heterogenous and have not been fully characterized, and it is unclear which cell population is responsible for differentiation into MK lineages.

Aims: This study aimed to identify megakaryocytic progenitor cells in subcutaneous PAs that have high potential for differentiation into MKs.

Methods: To examine the identification of megakaryocytic progenitor cells in human subcutaneous PAs, commercial-available PAs (Cell Applications) were used. Expressions of surface markers were analyzed by flowcytometry. CD71(+/-) cells were selected by FACS and were then cultured in MK induction media (Matsubara et al, 2012 Methods Mol Biol) for 6 days. MKs were defined as large-sized CD41(+) cells by fluorescent single cell analyzer. Thrombopoietin (TPO) levels in culture media during MK differentiation were measured by ELISA.

Results: Human PAs expressed surface markers for mesenchymal cells, but not hematopoietic cells: CD34(-)/CD45(-)/CD41(-)/CD14(-)/CD31(-)/CD116(-)/CD235(-)/CD56(-)/CD29(+)/CD13(+)/CD73(+)/CD90(+)/CD44(+). These markers were relatively evenly expressed, although CD71 expression was observed only in approximately 40% of cells. Previous study showed that CD71 on PAs is involved in the mechanism of TPO production for MK differentiation. Thus, this study examined the MK differentiation from CD71(+/-) PAs. Frequency of MKs derived from CD71(+) PAs were higher than that from CD71(-) PAs (31% vs 5.5%, $P = 0.013$). Also, TPO secretion levels were higher in CD71(+) PAs than in CD71(-) PAs (31 vs 12 pg mL⁻¹, $P = 0.014$). Expression of TPO receptor c-MPL was not different between CD71(+/-) PAs.

Conclusion: CD71(+) PAs are considered to be one of the appropriate markers for megakaryocytic progenitor cells, based on their higher productive capacity of TPO and capability of differentiating into MKs.

Disclosure of Interest: None Declared.

PO387-WED

Platelet factor XIII: the rescue in the factor FXIII deficiency

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Background: Coagulation factor XIII (FXIII) circulates in plasma as a heterotetramer molecule FXIII A2B2 and in an active form FXIII A2 in the cells. FXIII A2 stabilizes fibrin and protects it from fibrinolysis. FXIII A2 is synthesized by megakaryocytes and monocytes/macrophages, whereas FXIII B is synthesized by hepatocytes. Platelets contained a large amount of FXIII A and the concentration is about 150-fold greater than that in plasma. Congenital FXIII A deficiency is a rare inherited disorder associated with bleeding diathesis and requires regular administration of concentrates FXIII or fresh frozen plasma. The origin of platelets FXIII A is partially identified however whether megakaryocytes (MKs) endocytosis FXIII from plasma is still unclear.

Aims: We investigated the synthesis and uptake of FXIII by human MKs differentiated ex vivo from circulating progenitor cells of a

patient with FXIII deficiency in order to evaluate their ability to restore FXIII contents in MKs and platelets.

Methods: The patient has a history of severe intracranial haemorrhage and frequent bleeding. FXIII activity and antigen were undetectable in plasma and platelet lysates. Haematopoietic stem cells from peripheral blood were isolated and differentiated into MKs. Purified human FXIII was added to the MKs growth medium.

Results: Mutation analysis identified two mutations: p.G421S and p.Y215*. Western blot analysis in plasma and platelet lysates demonstrated the absence of the subunit A. FXIII A was not detectable in MKs from the patient while it was in normal subjects. Adding purified FXIII to the culture medium, some of the MKs stained positive for FXIII due to the uptake of the exogenous FXIII.

Conclusion: Normal MKs synthesizes FXIII A. MKs from a severe FXIII deficient patient cannot synthesize even though they preserve the ability to endocytosis exogenous FXIII from the culture medium. Furthermore it could be considered a 'rescue' mechanism of intraplatelet FXIII levels after administration of FXIII concentrates.

Disclosure of Interest: None Declared.

Microparticles III

PO388-WED

Rac1 regulates platelet microparticles formation and rheumatoid arthritis deterioration

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Background: Platelets circulate in blood and play essential roles in hemostasis, thrombosis and wound repair. Recently, accumulating evidence shows platelets also critically involve in the inflammation. Platelet microparticles (PMP), a plasma membrane vesicles derived from activated platelets, are reported to stimulate synovial cells to dump activating cytokines into the joint space thereby amplify inflammation in rheumatoid arthritis (RA) patients. However, the critical regulation molecules of PMP formation and its roles in RA deterioration still unclear.

Aims: Our study aimed to elucidate the critical regulation mechanism of PMP formation and identify the corresponding drug targets for prevention of RA deterioration.

Methods: Collagen-induced arthritis (CIA) mouse model, purified fibroblast-like synovial cells (FLS), and the technologies of FACS and hematoxylin and eosin staining were performed to evaluate the key regulation factors for PMP involving in RA.

Results: By screening the inhibitors, we found that either Rac1 specific inhibitor NSC23766 or Rac1 deficiency dramatically suppresses collagen-induced PMP formation. As expected, culture of mouse FLS with platelets caused PMP formation, which was also suppressed by NSC23766. *In vivo*, NSC23766 significantly reduced hind paw thickness and ankle stiffness and incidence of arthritis. Histological results demonstrated that spleens from NSC23766-treated CIA mice were less swollen than PBS control group, and contained normal size white pulps. Compared with control, NSC23766 suppressed the levels of PMP in the CIA mice peripheral blood. More interestingly, NSC23766 was obviously able to relieve synovial hyperplasia and protect cartilage from erosion.

Conclusion: In summary, the study presented here demonstrates that Rac1 is a key regulator for RA deterioration through aggravating the release of PMP. Therefore, Rac1 inhibition is a new strategy to alleviate RA.

Disclosure of Interest: None Declared.

PO389-WED

Alterations in the extracellular vesicles properties indicate hypercoagulable state in patients with acute myeloid leukemia

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Background: Patients with acute myeloid leukemia (AML) may suffer from venous thromboembolism (VTE) despite common thrombocytopenia. Extracellular vesicles (EVs) bearing tissue factor (TF) are shed from normal and pathologic cells and express coagulation proteins and antigens, reflecting their cellular origin.

Aims: This study was aimed to explore whether circulating EVs can predict thrombogenicity at diagnosis, post-chemotherapy nadir and remission in AML patients.

Methods: Blood samples were collected from healthy controls and patients with newly diagnosed AML at three time points: diagnosis, nadir and remission. EV concentration, cell origin and expression of coagulation proteins were analyzed by FACS. Procoagulant activity of EVs and their expression on endothelial cells was assessed using Factor Xa chromogenic assay.

Results: Forty two AML patients were enrolled in the study. Percentage of endothelial EVs was higher in patients compared to controls (28.4 vs. 7.5; $P < 0.05$), while platelet EV levels were lower compared to controls at all the three time points (10.3 vs. 30.7; $P < 0.05$, 7.9 vs. 30.7; $P < 0.001$, 9.4 vs. 30.7; $P < 0.01$, respectively). Percentage of TF-labeled EVs was higher at diagnosis (14.8 vs. 3.6; $P < 0.05$) and in remission (9.8 vs. 3.6) compared to controls. EV TF/TFPI ratio was found to be significantly elevated in patients at 3 time points compared to controls (AML I-10.34; AML II- 6.823; AML III-10.10; controls - 0.3499, $P < 0.0001$). EV procoagulant activity expressed as TF arbitrary unit was also increased (AML I-92.54; AML II-63.01; AML III-135.0; controls 7.953). Unlike controls' EVs, patients' EVs increased endothelial cell thrombogenicity.

Conclusion: EVs of AML patients express TF and induce endothelial procoagulant activity. Increased levels of endothelial EVs and their procoagulant activity may indicate a vascular injury associated with a hypercoagulable state in AML.

Disclosure of Interest: None Declared.

PO390-WED

Effect of acetylsalicylic acid on the concentration of monocyte microparticles coexpressing tissue factor in patients with diabetes mellitus type 2

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Background: Diabetes mellitus type 2 (DMT2) is a metabolic disease characterized by chronic hyperglycemia and hyperinsulinemia, leading to a prothrombotic condition in the individuals affected. The higher circulation tissue factor (TF) levels observed in DMT2 are a consequence of the chronic inflammatory state. The main sources of TF in the organism are monocytes, and in the circulation the main TF fraction is associated with the monocytes microparticles (MPs). Acetylsali-

cyclic acid (ASA) can be used in diabetics in order to help prevent atherothrombotic events.

Aims: The aims of this study were to determine the intra-assay variability of flow cytometry, used to quantify the MPs, as well as to evaluate the effect of low doses of ASA on the concentrations of monocyte MPs coexpressing TF (MMPs TF) in patients with DMT2.

Methods: Samples were collected from 25 patients with DMT2, before and after 15 consecutive days of using ASA at a dosage of 100 mg day⁻¹. The monocyte MPs coexpressing TF were identified and quantified by flow cytometry. All the samples were analyzed in duplicate, with the objective of determining the intra-assay coefficient of variation (CVI).

Results: The CVI value obtained for quantification of the MMPs TF was 15.10% for an average of 5.8 MPs μL^{-1} . For samples with concentrations of <4 MPs μL^{-1} , the CVI value was significantly higher (24.97%). There were significant ($P = 0.01$) decreases in the concentrations of MPs following the use of ASA, with values of 5.4 MPs μL^{-1} (range 1.1–16.7 MPs μL^{-1}) before the treatment and 3.8 MPs μL^{-1} (range 0.2–15.7 MPs μL^{-1}) after the treatment. The use of ASA promoted reductions in the concentrations of the MMPs TF in all the patients with ≥ 7.0 MPs μL^{-1} ($n = 11$).

Conclusion: The CVI values obtained here using flow cytometry were comparable to those reported in the literature for MPs derived from platelets. Low doses of ASA had a significant effect on reducing the concentrations of MMPs TF in patients with DMT2.

Disclosure of Interest: None Declared.

PO391-WED

Low microparticle-associated tissue factor activity in patients with persistent lupus anticoagulant and a history of thrombosis

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Background: The presence of antiphospholipid antibodies (APLA) is a strong risk factor of thrombosis. Previous studies demonstrated that APLAs induce the expression of tissue factor (TF), the main initiator of the coagulation cascade, on circulating monocytes. We hypothesized that APLAs might also lead to the generation of prothrombotic TF-bearing microparticles (MPs).

Aims: To investigate whether MP-associated TF activity is elevated in a well defined group of lupus anticoagulant (LA) positive patients with a history of thrombosis in comparison to healthy controls. Correlations between MP-TF activity, anti-beta-2-glycoprotein I (anti- β 2GPI) and anticardiolipin antibodies (ACA) in LA positives were investigated as well.

Methods: One-hundred-thirteen consecutive adult patients (median age: 39.7 years, interquartile range (IQR): 29.9–54.7; 87.1% females) persistently positive for LA and a history of thrombosis (85% venous thrombosis, 22% arterial thrombosis, some patients had venous and arterial events) and healthy age- and sex- matched controls ($n = 30$, median age: 43.4 years, IQR:40.5–45.9; 76% females) were included into this study. MP-TF activity was determined with a factor Xa generation assay and anti- β 2GPI and ACA antibodies by enzyme-linked immunoassays.

Results: MP-TF activity did not differ between LA positive patients with a history of thrombosis (median: 0.04 pg mL⁻¹, IQR: 0.00–0.11) and healthy controls (median: 0.06, IQR:0.00–0.10, $P = 0.896$). No correlation was found between MP-TF activity and anti- β 2GPI IgG ($r = 0.02$), anti- β 2GPI IgM ($r = -0.10$), ACA IgG ($r = 0.10$), or ACA IgM ($r = -0.11$).

Conclusion: We found very low MP-TF activity levels in a large group of patients with persistently positive LA and a history of thrombosis and no correlation with anti-β2GPI and ACA antibodies. Our data indicate that TF-bearing MPs do not contribute to the prothrombotic state of patients with APLAs.

Disclosure of Interest: None Declared.

PO392-WED

Meal intake increases circulating procoagulant microparticles in patients with diabetes mellitus

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Background: Diabetes mellitus (DM) is associated with prothrombotic alterations, and postprandial hyperglycemia is an independent risk factor for cardiovascular complications

Aims: To investigate whether a carbohydrate rich meal alters circulating microparticles (MPs) and their procoagulant activity in DM patients

Methods: Patients with type 1 (T1DM, n = 11) and type 2 (T2DM; n = 9) DM were studied before and 90 min after a standardized meal without premeal insulin. MPs in plasma derived from platelets (PMPs), endothelial cells (EMPs) or monocytes (MMPs) were measured by flow cytometry. MP-induced thrombin generation in plasma was assessed by a calibrated automated thrombogram

Results: Fasting MPs did not differ significantly between T1DM and T2DM. Meal intake increased PMPs expressing phosphatidylserine, P-selectin and/or tissue factor in T1DM while only PMPs expressing tissue factor increased significantly in T2DM. EMPs expressing E-selectin and MMPs expressing tissue factor were increased postprandially in both groups but there were no increments in EMPs expressing phosphatidylserine or tissue factor. Meal intake increased MP-induced thrombin generation similarly in T1DM and T2DM with increased endogenous thrombin potential ($P = 0.02$) and peak thrombin ($P = 0.03$), and shortened time to peak ($P = 0.02$). Phosphatidylserine inhibition by lactadherin abolished MP-induced thrombin generation while an anti-tissue factor antibody had no effect

Conclusion: Meal intake increased MPs differentially in patients with T1DM and T2DM. The procoagulant potential of MPs, however, increased similarly in T1DM and T2DM patients, and was related to phosphatidylserine expression and negatively charged MP surfaces rather than tissue factor. Procoagulant MP release may contribute to the increased cardiovascular risk associated with postprandial hyperglycemia

Disclosure of Interest: None Declared.

PO393-WED

ATRA modulates the emission of extracellular vesicles – implications for intercellular communication between acute promyelocytic leukemia and endothelial cells

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Background: Acute promyelocytic leukemia (APL) is characterized by fusion between promyelocytic leukemia gene (PML) and retinoic acid receptor α (RAR α), leading to a block in myeloid differentiation, oncogenic transformation and leukemic growth. The associated prominent vascular events include disseminated intravascular coagulation (DIC), expression of tissue factor (TF) and poorly studied bone marrow angiogenesis. Targeting PML-RAR α oncogene with all-trans retinoic acid (ATRA) and arsenic trioxide (ATO), combined with chemotherapy, leads to APL remission and a reversal of some of the vascular alterations. It remains unknown whether oncogene-dependent deregulation of intercellular exchange of extracellular vesicles (EVs) affects vascular events in APL.

Aims: In this study, we explored the role of PML-RAR α and ATRA in the regulation of EV-mediated communication between APL cells and the vascular system.

Methods: NB4 cells derived from an APL patient with t(15;17) were studied for EV cargo, membrane and molecular transfer to endothelial cells (HUVEC). Fluorescent dyes (PKH26), flow cytometry, western, PCR, and MTS assays were used to detect EV effects.

Results: NB4 cells shed EVs in a manner influenced by exposure to ATRA, suggesting the role of PML-RAR α oncogene in vesiculation. Leukemic EVs can modulate the survival and coagulant phenotype of endothelial cells. PML-RAR α is excluded from the EV cargo and intercellular transfer, while ATRA regulates the expression of multiple angiogenesis-related transcripts and proteins in both NB4 cells and their EVs (including VEGF, TF and IL-8).

Conclusion: PML-RAR α may indirectly influence the EV-mediated intercellular communication, angiogenesis and coagulopathy in APL. EV profiles may reveal the effects of ATRA on cancer cells.

Disclosure of Interest: None Declared.

PO394-WED

Clinical significance of circulating microparticles in Philadelphia myeloproliferative neoplasms (MPN)

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Background: Microparticles (MPs) are small membrane vesicles that are classified as platelet-derived MPs (PMPs), endothelial MPs (EMPs), red blood cell MPs (RMPs) and tissue factor MPs (TF+MPs) based on their origins. Philadelphia chromosome-negative myeloproliferative neoplasms (Ph-MPN) are disorders characterized by abnormal hematopoiesis, thrombosis and JAK2V617F mutation. Although MPs are considered as biomarkers reflecting procoagulant state in cancer patients, whether they exist in the patients with Ph-MPN remains unclear.

Aims: To measure the variation of the four types of MPs in the patients with MPN and to figure out their correlations with JAK2V617F mutation and some clinical complications.

Methods: Ninety-two patients with MPN were enrolled in this study, including 60 essential thrombocythemia (ET), 20 polycythemia vera (PV), and 12 primary myelofibrosis (PMF). Plasma samples were measured by flow cytometry for RMPs, PMPs, TF+MPs and EMPs with phycoerythrin conjugated monoclonal antibodies.

Results: (1) Levels of RMPs, PMPs, EMPs and TF+MPs in patients with Ph-MPN were all significantly higher than control group ($P < 0.05$). MPs in PMF group demonstrate higher than PV group ($P < 0.05$). (2) Ph-MPN patients with thrombosis complication showed higher levels of MPs than those without thrombosis complication ($P < 0.01$). (3) Ph-MPN patients with splenomegaly showed higher levels of MPs than those without splenomegaly ($P < 0.01$). (4) All four types of MPs in the JAK2V617F mutation group were higher than the group in which those patients without mutation ($P < 0.05$).

Conclusion: Ph-MPN patients reveals higher levels of all four types of MPs than controls, especially in patients complicated with thrombosis or splenomegaly. MPs in PMF patients possess more obvious increase than PV and ET groups. Patients with JAK2V617F mutation showed higher levels of the four types of MPs than that without mutation. MPs play an crucial role in the pathogenesis of Ph-MPN, and the MPs released may promote the formation of thrombosis.

Disclosure of Interest: None Declared.

PO395-WED

Platelet microvesicles in vascular inflammation

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Background: Microvesicles are gathering increasing attention as mediators of cell communication and as integral effectors of disease. Platelets present a major source of microvesicles and release these microvesicles either spontaneously or upon activation. Platelet microvesicles (PMVs) retain many features of their parent cells and have been shown to exert modulatory effects on vascular and immune cells.

Aims: We hypothesize that PMVs interact with vascular smooth muscle cells (SMCs) and modulate their function in the context of vascular remodeling.

Methods: PMVs were isolated from stored human platelet-rich plasma by serial centrifugation steps. PMVs were quantified and characterized by flow cytometry using annexin A5/phosphatidylserine and antibodies against CD41a/GP11b. Size calibrated micro beads were used to quantify the absolute amount of PMVs/mL. Cell migration experiments were performed using a chemotaxis μ -slide. Proliferation of SMCs was measured by the BrdU-cell proliferation kit. Platelet receptors implicated in PMV-SMC interaction were identified by blocking antibodies.

Results: In the presence of PMVs a gradient was generated that resulted in significant directed migration of the SMCs. Both, the total displacement distance and the velocity of movement of SMCs were significantly increased compared to the control condition without PMVs. The PMV binding to SMCs was specifically abrogated with the integrin $\alpha_{IIb}\beta_3$ inhibitor (integrilin) indicating an integrin-dependent mechanism of interaction. A proliferative effect on SMCs was measured after 48 h after incubation with PMVs and this proliferation relied on interactions via integrin $\alpha_M\beta_2$, CD40 and on P-selectin.

Conclusion: Isolated PMVs have shown to exert an immunomodulatory activity on various cell types. The present data indicate a role of PMVs in SMC chemotaxis and proliferation, which might contribute to vascular atherogenesis, in particular vascular remodeling.

Disclosure of Interest: None Declared.

PO396-WED

Impact of a fat-tolerance test on plasma concentration and size distribution of extracellular vesicles assessed by nanoparticle tracking analysis

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Background: Extracellular vesicle (EV) is a collective term for nano scale double membrane particles derived from activated and apoptotic cells. Plasma concentrations of EVs are affected by pre-analytical variables. Nanoparticle tracking analysis (NTA) is superior to flow cytometry, enabling a more precise count of EVs (50 nm–1 μ m). No previous study has, to our knowledge, studied the impact of the postprandial state on plasma concentration of EVs using NTA.

Aims: To investigate whether plasma EV concentration and size distribution is influenced by postprandial lipemia in healthy volunteers, using NanoSight NS300.

Methods: We recruited 23 healthy volunteers from the general population. After 12 h overnight fasting, they were subjected to a standardized fat tolerance test (1 g kg⁻¹ body weight) followed by blood sampling every second hour for 8 h. EVs were isolated from citrated platelet free plasma by centrifugation at 20,000 g for 30 min and measured by NanoSight NS300. The study was approved by the regional committee for research ethics and all subjects gave informed written consent.

Results: The fat-tolerance test caused an expected increase in postprandial lipoproteins, which peaked at 4 h and declined thereafter to baseline values within 8 h. Plasma concentrations of EVs increased from median 12.8 $\times 10^{10}$ mL⁻¹ (9.9-28.4) (interquartile range [IQR]) in the fasting state to median 14.3 $\times 10^{10}$ mL⁻¹ (9.9-19.0) (IQR) 2 h after a high fat meal (+12%, $P = 0.59$), followed by decrease (-4 to -17% from the fasting state) at hour 4, 6 and 8 (P for trend < 0.05). Dividing EVs into size categories revealed that only the smallest EVs (< 100 nm), accounting for the largest fraction of total EVs, showed this pattern.

Conclusion: Our findings showed that a high fat meal had a modest, but significant impact on plasma concentration of EVs, suggesting that the fasting status should be considered when measuring plasma EVs.

Disclosure of Interest: None Declared.

PO397-WED

Dynamics of interactions among platelet microparticles, and inflammatory, hemostatic and oxidative biomarkers in elderly individuals with and without cognitive impairment

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Background: Potential causes of platelet activation include inflammatory diseases, oxidative stress, hyperhomocysteinemia, endothelial cell damage, thrombin generation and others. This activation leads to a vesiculation process forming platelets microparticles (PMPs) and

increased PMPs levels have been observed in Alzheimer disease (AD) patients.

Aims: To compare the dynamics of interaction between PMPs and levels of soluble tumor necrosis factor alpha receptors 1 and 2 (sTNFR1; sTNFR2), intercellular adhesion molecule-1 (sICAM-1), Homocysteine (Hcy), FV and thiobarbituric acid reactive substances (TBARS), in DA patients and in subjects without cognitive impairment (controls).

Methods: To compare the dynamics of interaction between PMPs and biomarkers in both groups by using a biomarkers network based on the significant Spearman correlation coefficients to categorize correlations strength as negative ($r < 0$), moderate ($0.36 > r < 0.67$) and strong ($r > 0.68$). Cytoscape software was used for network arts. PMPs and biomarkers were determined in 81 elderly subjects with AD and 31 controls. PMPs phenotype was analysed by flow cytometry using annexin V and fluorochrome-labeled monoclonal antibodies against platelet surface proteins. sTNFR1, sTNFR2 and sICAM-1 were determined by ELISA; FV by a prothrombinase - based assay; oxidative stress by TBARS and Hcy by HPLC.

Results: A clear connection between PMPs and sTNFR2, sICAM-1, TBARS and FV was observed in the network for AD, whereas in the control group those connections were lost, signaling to the important role of the inflammatory and hemostatic factors in this disease. It was the first attempt made in identifying PMPs association with abnormalities related to inflammatory and hemostatic systems.

Conclusion: Concerning to the dynamics of interaction, DA patients showed a peculiar profile with significant connections from moderate to strong between PMPs and biomarkers, which did not occur in the control group.

Support: CAPES, CNPq and FAPEMIG-Brazil.

Disclosure of Interest: None Declared

Non-vitamin K antagonist oral anticoagulants III

PO398-WED

Performance of three functional assays for the measurement of dabigatran plasma concentrations

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Background: Dabigatran etexilate (DE) is now routinely used to treat a variety of patient conditions. DE is converted in dabigatran, a part of which being then glucuro-conjugated. Both free and conjugated forms exhibit the same anticoagulant activity, combined as total dabigatran (TD). Measurement of TD concentration is not recommended in routine practice, but can be helpful in emergency situations. An automated user-friendly assay can efficiently replace the cumbersome HPLC-MS/MS reference method.

Aims: To compare results generated by 3 different automated functional assays in 87 plasma samples obtained from patients receiving DE and assess accuracy vs. HPLC-MS/MS reference method.

Methods: STA[®]-ECAII (Diagnostics Stago, Asnières s/ Seine, France) (ECA) and Hemoclot thrombin inhibitor (Hyphen Biomed, Neuville sur Oise, France) (HTI) were performed on a STA-R[®] analyser (Diagnostics Stago) in addition to a homemade diluted thrombin time (dTT) test carried out on an ACL[®] Top analyser (Werfen, Bedford, MA, USA) in routine conditions. The HPLC-MS/MS assay for total dabigatran (MSTD) was performed by Nuvisan Pharma Services (Neu Ulm, Germany).

Results: MSTD values ranged from 14 to 476 ng mL⁻¹. Best correlation between methods was obtained between ECA and dTT ($R^2 = 0.983$), followed by similarly accurate results when comparing ECA or dTT to MSTD ($R^2 = 0.979$ and $R^2 = 0.973$ respectively). The

weakest correlation was observed between HTI and MSTD ($R^2 = 0.905$), mainly due to discordant values below 100 ng mL⁻¹ ($R^2 = 0.487$), a range where ECA and dTT remained highly correlated ($R^2 = 0.973$, $n = 26$).

Conclusion: This study confirms the good overall correlation between results obtained with the 3 different functional assays and the global accuracy of results when compared with the MSTD reference method. However, appropriate choice of the functional assay is of importance for the accurate assessment of dabigatran plasma concentration, especially for low dabigatran plasma concentrations (<100 ng mL⁻¹).

Disclosure of Interest: G. Freyburger Consultant for: Stago, Hyphen, Werfen, Boehringer Ingelheim, D. Girard: None Declared, A.-L. Vervaeke: None Declared, M. Rousset: None Declared, S. Labrousse: None Declared.

PO399-WED

Direct oral anticoagulants (DOAs) plasma levels in patients followed by a single center

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Background: Dabigatran, Rivaroxaban and Apixaban, three direct oral anticoagulant (DOA), are administered at fixed dose with different posology in relation to clinical indications, individual characteristics and renal function. In many studies large inter and intra individual variability has been shown both at trough (Ctrough) and at peak level (Cpeak).

Aims: Assess inter-individual variability levels in patients afferring to our center and treated with Dabigatran or Rivaroxaban.

Methods: 87 patients were enrolled and plasma samples were collected at Ctrough and Cpeak after one month from starting DOAs. Dilute thrombin time was used for Dabigatran, antiXa activity for Rivaroxaban. Assays in patients treated with apixaban were not performed. All patients underwrote an informed consent.

Results: Wide Ctrough and Cpeak inter-individual variability were found for both doses of the two drugs. For Dabigatran 110 mg 27 patients were studied: Ctrough was 47 (range 5-153) and Cpeak 167 (range 85-263); for Dabigatran 150 mg (13 patients) Ctrough was 77 (range 40-270) and Cpeak 198 (range 102-600). For Rivaroxaban 20 mg (43 patients) Ctrough was 56 (range 39-163) and Cpeak 212 (range 77-469). Only four patients received Rivaroxaban 15 mg (patient n.1 Ctrough 0 and Cpeak 132, n.2 Ctrough 63 and Cpeak 313, n.3 Ctrough 126 and Cpeak 291, n.4 Ctrough 42 and Cpeak 264).

Conclusion: Our data confirm the high inter-individual variability of the concentrations of both drugs. It could be useful assess the meaning to be given, in terms of efficacy and safety, to the extreme values of concentration (lower than 50 or higher than 200).

Disclosure of Interest: None Declared.

PO400-WED

Direct oral anticoagulants and surgery: our experience

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Background: The oral direct anticoagulants have emerged recently as an alternative to vitamin K antagonists (VKA).

Aims: We describe our experience in patients treated with Direct Oral Anticoagulants (DOAs) and who were undergoing surgery.

Methods: From June 2013 to December 2014, 165 patients (87 males, 78 females), aged 18–95 (mean 71) received DOAs. In 18 months, 9 patients were undergoing surgery.

Results: Patient number 1 was a male, 72 years old, treated with Dabigatran 150 mg bid for AF. He undergoing major orthopaedic surgery for hip fracture 24 h after the last dose. Laboratory data showed normal aPTT and Thrombin Time (TT).

Patient number 2 was a male, 85 years old, treated with Dabigatran 110 mg bid for AF. He undergoing emergency surgery for acute cholecystitis, 16 h after the last dose of DOA.

Patient number 3 was a female, 61 years old, treated with Rivaroxaban 20 mg bid for AF. The patient had a history of cerebral venous thrombosis and was suffering from thrombophilia (heterozygous mutation of prothrombin). She undergoing mastectomy for breast cancer, 48 h after the last dose of Xarelto. Before surgery she began briding with enoxaparin. All of these patients that undergoing major surgery were free of events.

Patient number 4 was a male, treated with Apixaban 5 mg bid for AF, undergoing tooth extraction. Patients 5–9 underwent surgery for cataract (2 patients treated with Dabigatran, 1 patient with Rivaroxaban and 2 patients with Apixaban). In patients 4–9, in which the interventions did carry no clinically important bleeding risk and in which adequate local haemostasis was possible, the procedures were performed at trough concentration of the DOAs (12–24 h after the last intake, depending on bid or qd dosing). No events was observed.

Conclusion: The short half-life of DOAs allows to make surgeries after a brief suspension of anticoagulant in patients with normal renal function.

Disclosure of Interest: None Declared.

PO401-WED

Dilute russell viper venom time, a partial solution for screening and concentration determination of direct oral anticoagulants

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Background: The introduction of direct oral anticoagulants (NOACs) in clinical practice led to the need of reliable screening and concentration determining tools of these anticoagulants. Specific assays are available to establish the concentration of either direct thrombin- or direct factor Xa- inhibitors, but these cannot easily be used in daily practice. The dilute Russell Viper Venom time (dRVVT) could be a good candidate for routine use because it can be used for both types of inhibitors on most coagulation analysers.

Aims: To establish whether dRVVT is suitable for NOACs screening and concentration determination.

Methods: The effect of the NOACs dabigatran, rivaroxaban and apixaban was tested on four commercial dRVVT assays using spiked normal pooled plasma. Also, dRVVT was measured in samples from patients receiving one of the NOACs to study the usability of this assay in patient care.

Results: All four dRVVT assays showed linear dose-response curves for all tested NOACs and with sufficient prolongation in the therapeutic range. Prolongation at the upper limit of the therapeutic range was for dabigatran 80–150 s, rivaroxaban 70–120 s and apixaban 17–38 s. In patient samples containing dabigatran (n = 31), the dRVVT proved to be suitable for screening and concentration determination ($Y = 0.9X - 4.3$ comparison with LC-MS/MS). With rivaroxaban (n = 21), screening with dRVVT is possible, but concentration determination is too variable ($Y = 0.9X + 61$ compared to LC-MS/MS). With apixaban (n = 5) sometimes no prolongation of dRVVT was observed.

Conclusion: Based on *in vitro* studies dRVVT seems a good screening and concentration determination assay for all three tested NOACs. However, *in vivo* results show only promising results as a screening assay for dabigatran and rivaroxaban, and concentration determination tool for dabigatran in the therapeutic range. The dRVVT test is likely to demonstrate the presence of supratherapeutic concentrations with all three NOACs.

Disclosure of Interest: A. Stroobants Grant/Research Support from: dRVVT reagents were provided by Stago, IL and Siemens, Speaker Bureau of: Stago, E.-J. van den Dool: None Declared, M. Heckman: None Declared, M. Coppens Grant/Research Support from: Boehringer-Ingelheim, Bayer, Bristol-Myers Squibb, Pfizer, and Daiichi Sankyo., Consultant for: Boehringer-Ingelheim, Bayer, Bristol-Myers Squibb, Pfizer, and Daiichi Sankyo., Speaker Bureau of: Boehringer-Ingelheim, Bayer, Bristol-Myers Squibb, Pfizer, and Daiichi Sankyo., A. Sturk: None Declared.

PO402-WED

Drug interactions of newer oral anticoagulants dabigatran, rivaroxaban, and apixaban with routinely used nonanticoagulant/antiplatelet drugs

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Background: The newer non-vitamin K antagonist oral anticoagulant drugs (NVKOCs) such as dabigatran(D), apixaban(A) and rivaroxaban(R) are simultaneously used with several routinely used drugs. We hypothesized that some of the commonly used drugs may modulate the effects of NVKOCs.

Aims: This study aims to determine anticoagulant and bleeding effects of the NVKOCs with such drugs as alendronate sodium, chondroitin sulfate, hydrocodone-acetaminophen, klonopin, penicillin, tacrolimus, tramadol chlorhydrate, and tranexamic acid(TA).

Methods: These drugs were supplemented into citrated plasma at projected therapeutic ranges. Tests included PT, APTT, dRVVT, TT, HepTest, anti-Xa and anti-IIa studies were performed with D, A and R alone and with the routinely used drugs. For bleeding studies a rat tail transection model was used with D alone and D followed by TA.

Results: All of the NVKOCs produced assay dependent anticoagulant and antiprotease effects. R and A did not exhibit any interactions when combined with any of the routinely used drugs. However D at a fixed concentration of 1 $\mu\text{g mL}^{-1}$ combined with the commonly used drugs at a fixed concentrations produced augmented assay-dependent anticoagulant and antiprotease activity. The most pronounced interaction was noticed with tacrolimus (111% difference in PT, 231% difference in APTT, and 46% difference in anti-IIa assay), followed by tramadol (57% difference in PT and 54% difference in Anti-IIa assay). In the rat tail transection bleeding model, there was a significant difference ($P = 0.03$, $\alpha=0.05$) between the bleeding time with D (100 $\mu\text{g kg}^{-1}$) alone (13.1 $\pm$ 1.5 min) intravenously compared to D with TA (10 mg kg^{-1}) (10.3 $\pm$ 1.8 min).

Conclusion: These results warrant a review of post-marketing surveillance on the reported bleeding in patients concomitantly treated with NVKOCs and the reported routinely used drugs. Furthermore, these observations underscore the need to screen other commonly used drugs and supplements for their potential interactions with NVKOCs.

Disclosure of Interest: None Declared.

PO403-WED

Unexpectedly stable dabigatran levels in anticoagulation clinic patientsMcGlasson DL¹ and Fritsma GA²¹59th Clinical Research Division, United States Air Force, San Antonio; ²Fritsma Factor, Birmingham, AL, USA**Background:** The dabigatran effect is rapid and its half-life is 12–17 h. Experts believe that missed doses may lead to thrombosis.**Aims:** We evaluated plasma dabigatran levels on anticoagulation clinic patients monthly for 6 months.**Methods:** Subjects took 150 mg BID beginning one month prior. All subjects were > 18 years old and had creatinine clearances > 30 mL min⁻¹. No subjects were excluded for parallel medications or pre-existing conditions. We enrolled 64 males, mean age 77, and 38 females, mean age 76. Mean CHAD2DS2-VASc score was 3.2; mean BMI 29.8. We assayed specimens from 72 subjects who completed the six-month cycle and 30 healthy controls taking no medication. We collected a 3.2% sodium citrate specimen from each subject on a monthly date within ±5 days of the start date at a time convenient to the subject. We did not monitor the time subjects took medication. We prepared platelet poor plasma by immediate centrifugation and stored at -70°C. We assayed specimens using the APTT (APTT Automated, Stago); Ecarin Chromogenic Assay (ECA-T, Stago); Hemoclot Thrombin Inhibitor (HTI, Aniaira); and Prothrombinase-induced clotting time (PiCT, Enzyme Research Laboratory). Testing was performed on a Stago STAR-Evolution coagulometer.**Results:** Reference intervals were computed from controls for the APTT (mean 30.7 s; ±2SD: 26.1–35.3 s); PiCT (mean 40.1 s; ±2SD: 29.4–50.7 s). Dabigatran subjects' APTT range was 26.2–300 s, mean 53.7 s (> 300 was assigned 301 s); PiCT range was 60.8–301 s, mean 186.7 s; ECA-T range: 10–950 ng mL⁻¹, mean 177.6 ng mL⁻¹; HTI range 0.0–770 ng mL⁻¹, mean 185.3. A two-factor ANOVA (dabigatran, time) with repeated measures on all tests revealed no significant difference between monthly results within subjects ($P = 0.234$) for any assay.**Conclusion:** Our data imply reproducibility in the absence of strict dosage time vs. specimen collection time. This unexpected consequence implies a need for prospective studies that determine dabigatran anticoagulant effect vs. time subsequent to dosage.**Disclosure of Interest:** None Declared.

PO404-WED

Successful use of hemodialysis in a dabigatran overdoseFreixo A¹, Ferreira A², Gonçalves L¹, Santos L^{2,3}, Carvalho M¹, Sarmiento A^{2,3} and Araujo F^{1,3}¹Department of Transfusion Medicine and Blood Bank;²Department of Infectious Diseases, São João Hospital;³Medicine Faculty, Oporto University, Porto, Portugal**Background:** Dabigatran etexilate is an oral direct thrombin inhibitor. When there is a need to reverse its anticoagulant activity there is a lack of experience.**Aims:** To describe a clinical case of the use of hemodialysis to treat a dabigatran overdose.**Methods:** An 81-year-old female with nonvalvular atrial fibrillation under dabigatran (75 mg bid), and moderate chronic renal impairment was admitted to our institution due to a profuse diarrhea, for the last two days. The laboratory tests showed hemoglobin 12.8 g dL⁻¹, platelet count 236,000 L⁻¹, CrCl 16 mL min⁻¹, aPTT 123 s (N: 24,2–36,4) and dabigatran concentration 1178 ng mL⁻¹.**Results:** Despite abnormal coagulation tests, a colonoscopy with biopsy was performed, with no bleeding complications, and an infectious/ischemic colitis was detected. In order to place a central venouscatheter and an arterial line, activated prothrombin complex concentrate (FEIBA) 50 IU kg⁻¹ was given, and the procedures were performed with no bleeding complications, although coagulation tests remained abnormal (aPTT 137 s; dabigatran 1047 ng mL⁻¹). As the patient became hemodynamically unstable and oliguric, continuous venovenous hemodiafiltration (CVVHDF) was performed, at a blood flow rate of 350 mL h⁻¹, for 21 h, and repeated 24 h later for 20 h. During CVVHDF, dabigatran concentrations reduced 75% in the first period and 62% in the next. The patient had no bleeding episodes during hospitalization.**Conclusion:** This case highlights three important issues when dealing with patients on dabigatran requiring invasive procedures: 1) low bleeding risk of endoscopy with biopsy, even with high levels of the drug; 2) the clinical success of FEIBA for catheter placement with no correlation with coagulation tests; 3) the efficacy of CVVHDF in removing the drug. Until the antidote is available, several established procedures can be safe and effectively used, in reversing the dabigatran anticoagulant activity.**Disclosure of Interest:** None Declared.

PO405-WED

Clinical impact of bleeding complications with direct oral anticoagulants for the prevention of venous thromboembolism in orthopaedic surgery: a systematic review and meta-analysis of randomized controlled trials

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Background: Direct oral anticoagulants (DOACs) have been proposed as alternatives to low molecular weight heparins (LMWHs) for the prevention of venous thromboembolism in orthopaedic surgery, but the clinical impact of post-surgical bleeding with their use has not been extensively evaluated.**Aims:** to estimate the clinical impact of DOACs-related bleeding, defined as the incidence of fatal bleeding and the case-fatality rate of major bleeding.**Methods:** We performed a systematic review and meta-analysis of phase II and III randomized controlled trials comparing the DOACs with LMWHs in patients undergoing major orthopaedic surgery (elective hip/knee replacement or hip fracture surgery). MEDLINE and EMBASE databases, supplemented with conferences abstract books and www.clinicaltrial.gov, were searched up to September 2014 week 1, without any language restriction. Two reviewers performed independent article review and study quality assessment. Data regarding major, fatal and intracranial bleeding were collected, in order to calculate the pooled relative risk (RR) and the case-fatality rate (CFR), with associated 95% confidence interval (CI).**Results:** We retrieved 23 studies (4 with dabigatran, 4 with apixaban, 5 with edoxaban and 10 with rivaroxaban), enrolling a total of 42037 patients. There was no statistically significant difference between DOACs and LMWHs in the risk of major bleeding (0.80% vs 0.74%; RR 0.99, 95%CI 0.79–1.24), fatal bleeding (0.02% vs 0.01%; RR 1.63, 95%CI 0.39–6.77) and intracranial bleeding (0% vs 0.01%; RR 0.33, 95%CI 0.03–3.18). These results were confirmed in several sensitivity and subgroup analyses. The weighted mean CFR of major bleeding was 4.4% (95%CI, 1.9–7.8%) for DOACs and 3.4% (95%CI, 1.1–6.9%) for LMWHs.**Conclusion:** Bleeding complications and the associated CFR during prophylactic anticoagulation in orthopaedic surgery were very low and not significantly different between DOACs and LMWHs.**Disclosure of Interest:** E. Tamborini Permunian: None Declared, W. Ageno Grant/Research Support from: Bayer HealthCare, Glaxo-SmithKline, Pfizer-BMS, Daiichi Sankyo and Boehringer Ingelheim,

Consultant for: Bayer HealthCare, Boehringer Ingelheim, Bristol-Myers Squibb-Pfizer and Daiichi Sankyo, F. Dentali Grant/Research Support from: Bayer HealthCare, Pfizer-BMS and Boehringer Ingelheim., Consultant for: Bayer HealthCare, Boehringer Ingelheim and Bristol-Myers Squibb-Pfizer, N. Riva: None Declared.

PO406-WED

Role of anticoagulant clinics in the management of patients on non-vitamin K antagonist oral anticoagulants (NOACs) at Cork University Hospital (CUH)

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Background: Non-vitamin K antagonist oral anticoagulants (NOACs) are a new class of drugs for anticoagulation treatment that provide a viable alternative to traditional vitamin K antagonists (VKA) such as Warfarin. NOACs have the advantages of predictable pharmacokinetics, fixed dosing regime, fewer drug interactions and so far no known food interactions. Increasing numbers of patients are prescribed NOACs. Unlike traditional anticoagulation, these patients are not referred to anticoagulation clinics for treatment management after initiation.

Aims: The aim of this clinical observational study is to ensure that patients switched from VKA to a NOAC are managed appropriately in terms of education, prescribing, administration and follow up.

Methods: Anonymized data were extracted from the nurse led NOACs clinic over a period of six months. Data included demographics, indication for anticoagulation, type of education patient received, patients' knowledge of NOAC administration and management. Results were analyzed using Microsoft Excel.

Results: A total of 44 patients, aged between 21 and 90 years, attended the clinic. 59% of patients had atrial fibrillation and were receiving NOACs for stroke prevention, 15% for venous thromboembolism (VTE), 24% for recurrent VTE. At the time of switching to a NOAC, 29.5% patients received education, of which 22.7% received written information but only 11% were given alert cards. 77% patients administered it correctly. 54.5% reported missing a dose and out of this 34% missed two or more doses since initiation. 89% patients did not know how to manage a missed dose.

Conclusion: This study demonstrates that patients are poorly educated which puts them at an increased risk of bleeding or thromboembolic events. There is a danger that oversimplification of treatment with NOACs may lead to adverse events which in turn may result in mistrust of these drugs. This risk could be counteracted by the establishment of NOACs Clinics providing appropriate education, management and follow up.

Disclosure of Interest: None Declared.

PO407-WED

Are non-VKA oral anticoagulants (NOACs) plasma concentrations associated with the risk of postoperative bleeding? Preliminary results from the real life cohort

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Background: The perioperative management (PM) of NOACs is controversial. The role of assessing NOACs plasma levels in order to ensure a safe use of these anticoagulants is still unknown.

Aims: To examine the association between NOACs plasma concentrations obtained before surgery and the risk of postoperative bleeding in the perioperative setting.

Methods: From June 2014 to December 2014 we have consecutively included patients treated with NOACs and referred to our Unit for PM. Management was performed following the PM guidelines from the Spanish Forum of anticoagulants and Anaesthesia. Bleeding events were classified following the ISTH criteria. Plasma concentrations were measured in the day of invasive procedure using the Technoclone anti-Xa assay from Technoclone (Vienna-Austria) for Rivaroxaban and Apixaban, and the Direct Thrombin Inhibitor Assay from IL (Bedford-MA-USA) for Dabigatran; in each case, specific calibrators were used. Patients were systematically followed 30 days after the surgical procedure.

Results: A total of 33 patients were recruited. Median age was 74 years (range: 61-94) and 17 (51.5%) were female. Among them, 8 patients received dabigatran, 20 rivaroxaban and 5 apixaban. As per the bleeding risk score, 57.5% of the patients had a HAS-BLED score >3, and 51.5% were considered high-risk procedures. Total bleeding events occurred in 17 patients (47.1% minor, 35.3% non-major clinically relevant and 17.6% major bleeding). The median plasma NOACs concentration was 29.2 ng mL⁻¹ (0.8-226 ng mL⁻¹), with eleven patients having levels > 30 ng mL⁻¹. No correlation was found between drug plasma concentration and major bleeding events. Major bleeding (n = 3) was probably more related to the risk of the procedure than to the NOACs plasma concentration.

Conclusion: In our cohort we found no significant association between NOAC plasma concentrations obtained before surgery with the risk of postoperative bleeding. In spite of that, this study will continue to reevaluate PM in high-risk procedures according to plasma NOACs levels.

Disclosure of Interest: None Declared.

PO408-WED

A novel approach to evaluating oral anticoagulants

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Background: A central challenge in identifying effective anticoagulants is to achieve a broad therapeutic window in which anticoagulant activity is not too high—which risks hemorrhage—or too low—which risks thrombosis. The Hill coefficient, n_H , which in this case is related to how much a change in drug concentration changes coagulation activity, may be a useful gauge of an anticoagulant's therapeutic window.

Aims: We sought to measure the Hill coefficient of available anticoagulants, and combinations thereof, to gain insight into their therapeutic windows.

Methods: We used a simple fluorometric *in vitro* assay to determine clotting activity platelet poor plasma after exposure to various amounts of anticoagulants.

Results: We find that there is a significant variation among the Hill coefficients (between about 1.5–4.5) among anticoagulants in our assay. Furthermore, we find both combinations of anticoagulants that have greater and lower Hill coefficients than the individual drugs.

Conclusion: Our work suggests that the Hill coefficient may be a valuable measurement of the therapeutic index of anticoagulants. Furthermore, a polypharmacological approach may be effective in managing clotting-associated risk.

Disclosure of Interest: None Declared.

PO409-WED

Clinical presentation and extent of clinical care of clinically relevant non-major bleeding events with apixaban and warfarin

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Background: In the AMPLIFY trial, apixaban, a direct acting oral anticoagulant (DOAC), proved to be non-inferior to low-molecular-weight heparin (LMWH)/warfarin for treatment of venous thromboembolism, and was associated with significantly less bleeding. More information is needed on the type and severity of bleeding with DOACs.

Aims: In this post-hoc analysis, we blindly classified the seriousness of clinical presentation and subsequent clinical course of all episodes of clinically relevant non-major bleeding (CRNMB) in the AMPLIFY trial.

Methods: Three clinicians classified all CRNMB episodes, first independently and then by discussion, using pre-defined criteria. A classification scheme was developed, reflecting the extent of clinical care. Events were assigned to category one if they were resolved spontaneously, and/or were reported retrospectively during the trial. Category two comprised events that could not be classified in any of the other three categories. The third category comprised events requiring an emergency department or medical visit and procedures or treatment to control the bleeding. Category four was reserved for bleeding events requiring hospitalization.

Results: Of the 311 CRNMB episodes classified, 100 were in the apixaban group and 211 in the LMWH/warfarin group. A more severe clinical presentation and extent of clinical care (category three or four) was observed in 25.0% of the apixaban-associated CRNMB and in 22.7% of the LMWH/warfarin-associated CRNMB (odds ratio 1.13, 95% CI 0.65 – 1.97).

Conclusion: The clinical presentation and subsequent extent of clinical care of CRNMBs associated with apixaban and with LMWH/warfarin were comparable.

Disclosure of Interest: S. Bleker: None Declared, A. Cohen Consultant for: Bayer, Boehringer-Ingelheim, BMS, Daiichi, GSK, Johnson and Johnson, Mitsubishi Pharma, Pfizer, Portola, Sanofi, Schering Plough, Takeda, X01, Paid Instructor at: Bayer, Boehringer-Ingelheim, BMS, Daiichi, GSK, Johnson and Johnson, Mitsubishi Pharma, Pfizer, Portola, Sanofi, Schering Plough, H. Büller: None Declared, G. Agnelli: None Declared, A. Gallus Consultant for: BMS, Pfizer, G. Raskob Consultant for: Daiichi Sankyo, Bayer, BMS, Boehringer Ingelheim, Isis Pharmaceuticals, Janssen Pharmaceuticals, Speaker Bureau of: Bayer, Daiichi Sankyo, BMS/Pfizer, Janssen, J. Weitz: None Declared, M. Curto Employee of: Pfizer Inc., M. Sisson Shareholder of: Pfizer Inc., Employee of: Pfizer Inc., S. Middeldorp: None Declared.

PO410-WED

Interference study of direct thrombin inhibitors and anti-XA inhibitors on hemostasis assays on a cobas t 411 system

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Background: Direct thrombin inhibitors bivalirudin, argatroban and dabigatran and factor Xa inhibitors rivaroxaban, apixaban and fondaparinux are known to impact some routine coagulation parameters. The influence of these drugs on assays with the new cobas t 411 system was investigated.

Aims: Study the influence of direct thrombin and anti-Xa inhibitors on routine hemostasis assays. Assess whether the aPTT or PT assays are suitable to screen for the presence of such anticoagulants.

Methods: Pooled plasma from healthy donors was spiked with one of the anticoagulants in 6 concentrations up to the maximum of its therapeutic range. Measurements of aPTT (3 reagents), PT, antithrombin (AT) and fibrinogen were performed on 6 days with single measurements each day or, if no influence was expected, with 1 triplicate measurement on a cobas t 411 platform (Roche Diagnostics) using reagents and control material according to the manufacturer's instructions. Deviations were compared to within run CVs.

Results: The CVs of the assays for repeatability were as follows (max. values): aPTT 1.6%, PT 1.1%, AT 5.4%, fibrinogen 13%. Increases at the upper limit of the therapeutic range compared to normal were with bivalirudin for aPTT 145–199%, PT 62%; argatroban aPTT 187–255%, PT 121%; dabigatran aPTT 152–205%, PT 61%; rivaroxaban aPTT 60–70%, PT 34%; apixaban aPTT 18–31%, PT 14%; fondaparinux aPTT 14–25%, PT 8.3%.

All aPTT reagents were very sensitive to the presence of thrombin inhibitors, less to rivaroxaban and least to apixaban and fondaparinux. PT was sensitive to thrombin inhibitors, less to Xa inhibitors. The curvilinear dose response curves complicated concentration determination. Thrombin inhibitors impacted AT and fibrinogen as expected, except that for AT no increase was observed in the presence of bivalirudin.

Conclusion: These effects must be taken into consideration when interpreting coagulation results of patients receiving these anticoagulants. APTT and PT can be considered to screen for the presence of those drugs.

Disclosure of Interest: A. Stroobants Grant/Research Support from: Roche diagnostics to laboratory for general clinical chemistry, W. van Dam: None Declared, B. Bakker: None Declared, E.-J. van den Dool: None Declared, M. Heckman: None Declared.

PO411-WED

Neutralization of bleeding induced by non-vitamin K oral anticoagulants by prothrombin complex concentrates alone and in combination with antifibrinolytic agents

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Background: Concerns remain over bleeding with direct factor Xa and thrombin inhibitors in cases of overdose or medical emergency.

Aims: This study tested the ability of PCCs to reverse bleeding induced by Non Vitamin K oral anticoagulants such as apixaban, rivaroxaban or dabigatran in a standardized rat tail resection model.

Methods: Individual groups of anesthetized rats (8–10) were anticoagulated with apixaban, rivaroxaban or dabigatran and subsequently treated

ted with vehicle, 3-factor (Profilnine), 4-factor (Beriplex) or activated 4-factor PCC (FEIBA). Five minutes post-PCC administration bleeding time (BT) was measured. Upon cessation of bleeding, blood samples were collected for ex vivo analysis.

Results: In comparison to saline control (6.8 ± 1.1 s) apixaban ($300 \mu\text{g kg}^{-1}$) prolonged BT (17.1 ± 5.6 ; $P = 0.024$). Beriplex at doses $\leq 10\text{U/kg}$ did not shorten BT while 10U/kg Profilnine did. FEIBA prolonged bleeding beyond that seen with apixaban alone (22.6 ± 11.4 and 37.5 ± 5.2 min for 5 and 10U kg^{-1} doses; $P < 0.001$ apixaban + 10U kg^{-1} FEIBA vs. apixaban). 100 and $300 \mu\text{g kg}^{-1}$ rivaroxaban prolonged BT (20.8 ± 2.5 , $P = 0.002$ vs. saline; 25.0 ± 10.0 min, $P < 0.001$ vs. saline). Profilnine was more effective than Beriplex or FEIBA in reversing rivaroxaban-induced bleeding. Dabigatran-induced bleeding was prevented by Beriplex or Profilnine but enhanced by FEIBA (50.3 ± 8.3 vs. 15.9 ± 1.2 min; $P < 0.001$). Treatment of apixaban or rivaroxaban anticoagulated rats with FEIBA + 10mg kg^{-1} EACA, produced BTs comparable to those of control rats (apixaban: 6.7 ± 5.0 min; rivaroxaban: 7.7 ± 6.4 min). Ex vivo analysis of blood samples did not show any relevance with the observed bleeding or its modulation.

Conclusion: PCCs are useful for neutralizing bleeding induced by direct factor Xa and thrombin inhibitors, however each PCC exhibits a distinct neutralization profile. Adjunct usage of clinically available fibrinolytic inhibitors such as EACA and tranexamic acid may improve effectiveness of the non-vitamin K oral anticoagulants associated hemorrhage reversal by PCCs.

Disclosure of Interest: None Declared.

PO412-WED

The dilute FIIx prothrombin time (DFIIX-PT) for assessment of dabigatran, rivaroxaban and warfarin effect in patient samples

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Background: Fiix-PT is a modified PT that is only sensitive to activable factors (F) II and X in test samples. In previous spiking experiments diluting the thromboplastin increased sensitivity of both the PT and Fiix-PT to direct (D) oral anticoagulants (OACs).

Aims: To test the sensitivity of dilute PT (dPT) and dFiix-PT to the influence of DOACs and warfarin in patient samples.

Methods: Citrated plasma samples from patients on dabigatran, rivaroxaban or warfarin were analysed with dilute thrombin time (dTT), anti Xa assay (aXa) or PT, respectively. Samples were then stored at -80°C until analysed with dPT and dFiix-PT, using 1:750 and 1:1156 thromboplastin dilutions, respectively, and dabigatran and rivaroxaban calibrated standard curves to convert results to ng/mL. The dTT and aXa were converted to ng/mL using dabigatran and rivaroxaban standards. For warfarin, the dPT and dFiix-PT were expressed in seconds and compared to original INR.

Results: The dTT ($y = 1.03x$, $R^2 = 0.94$) and aXa assays ($y = 0.73x$, $R^2 = 0.95$) correlated well with mass spectrometry in patient samples. In 28 dabigatran samples, using dFiix-PT a linear relationship with dTT was observed ($y = 0.98x$, $R^2 = 0.73$). Using the dPT, a linear relationship was also observed but with more scattering ($y = 1.32x$, $R^2 = 0.62$). In 19 rivaroxaban samples a linear relationship was observed when dFiix-PT was compared to the aXa assay ($y = 1.13x$, $R^2 = 0.91$) and when dPT was compared with aXa ($y = 1.57x$, $R^2 = 0.89$). In 48

warfarin patient samples (INR 1.0–4.7), both dFiix-PT and dPT results correlated linearly with INR, $y = 74.7$ $R^2 = 0.83$ and $y = 23.1x$, $R^2 = 0.79$, respectively. The dFiix-PT ratio was identical to the INR whereas the PT 1:750 ratio was not.

Conclusion: A single dilution of either dPT or dFiix-PT can be used to assess the warfarin effect and concentration of dabigatran and rivaroxaban. However, the dFiix-PT gave more precise results, possibly due to elimination of confounding factors by mixing in FII and FX deficient plasma.

Disclosure of Interest: L. Letertre: None Declared, B. Gudmundsdottir Shareholder of: Patent pending on Fiix PT, M. Skeppholm: None Declared, R. Malmstrom: None Declared, C. Francis: None Declared, P. Onundarson Shareholder of: Patent pending on Fiix PT.

PO413-WED

Evaluation of appropriateness of rivaroxaban prescription in a tertiary care hospital in Singapore

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Background: Rivaroxaban is a novel oral anticoagulant newly added into our hospital formulary. Inappropriate use and unfamiliarity with prescribing can potentially lead to serious adverse events.

Aims: We aim to determine the appropriateness of rivaroxaban prescription and review its efficacy and safety outcomes in our hospital.

Methods: We did a retrospective review of medical records in consecutive patients who were newly prescribed rivaroxaban from 1 June 2012 to 31 March 2013. Criteria to evaluate appropriate prescription were indications, dosing, use in the absence of significant drug interactions and contraindications. We defined overall appropriateness as compliance to all of the above criteria. Efficacy and safety outcomes were determined by occurrence of thromboembolic and bleeding complications respectively. Patients were followed for the period receiving rivaroxaban or up to one year, whichever was longer.

Results: 113 patients were evaluated (median age 69 years, interquartile range 62–77). 105 (92.9%) patients had appropriate indication for rivaroxaban use: 93 (82.3%) for atrial fibrillation and 12 (10.6%) for treatment of acute deep vein thrombosis/pulmonary embolism. 8 (7.1%) had off-label use (valvular atrial fibrillation, peripheral vascular disease, heart thrombus, isolated stroke, atrial flutter). Amongst patients with appropriate indications, 83 (79%) received correct dosing. 8 (7.6%) had no renal adjusted dosing, 8 (7.6%) had no renal function done, 1 patient had $\text{CrCl} < 15 \text{ mL min}^{-1}$ and 5 (4.8%) were under-dosed. The overall inappropriateness of rivaroxaban prescription was 26.5%. 104 patients were included in the outcome analysis. 2 (1.9%) had stroke and 7 (6.7%) had clinically relevant minor bleeding. There was no major bleeding event.

Conclusion: There was a high inappropriateness of rivaroxaban prescription, with incorrect dosing being the most common. However, thromboembolic and bleeding complications remained low. This study highlights the need to have hospital prescribing guidance and education.

Disclosure of Interest: None Declared.

PO414-WED

Idarucizumab antagonizes dabigatran-induced alterations on platelet and fibrin deposition in an in vitro thrombosis model with circulating human blood

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Background: Reversal of the antihemostatic action of dabigatran in emergency situations remains controversial. Idarucizumab, a new specific antidote for dabigatran, has demonstrated efficacy in healthy volunteers.

Aims: We compared the efficacy of idarucizumab with non-specific procoagulant strategies at reversing effects of dabigatran to gain a better understanding of their interactions with mechanisms of hemostasis.

Methods: Concentrations of dabigatran equivalent to the C_{max} (184 ng mL⁻¹) were added *in vitro* to blood aliquots from 11 healthy donors. We evaluated the modifications in different coagulation tests: 1) fibrin and platelet deposition on damaged vascular segments with whole blood under flow conditions at a shear rate of 600/s, 2) thrombin generation (TG) and 3) thromboelastometry (ROTEM). The efficacy of idarucizumab (0.3, 1 and 3 mg mL⁻¹) was compared with: prothrombin complex concentrates (PCC, 70 IU kg⁻¹), activated PCC (aPCC, 25 and 75 IU kg⁻¹), or rFVIIa (120 µg kg⁻¹).

Results: Idarucizumab completely reversed the alterations in fibrin coverage and platelet deposition induced by dabigatran. TG and ROTEM parameters also returned to normal values after idarucizumab. Reversal strategies with aPCC or PCC normalized and even over-compensated alterations in TG kinetics and partially improved ROTEM parameters. aPCC and PCC moderately improved the alteration in fibrin deposition, but levels did not return to baseline values. rFVIIa showed only moderate effects on some of the biomarkers.

Conclusion: Procoagulant concentrates over-compensated or partially restored the anti-hemostatic action of dabigatran, but results were not consistent for all the tests. In clear contrast with non specific procoagulant strategies, idarucizumab, the specific antidote to dabigatran, completely reversed all alterations in coagulation parameters evaluated in circulating human blood and restored all coagulation biomarkers to pre-treatment values. (Grants: SAF 2011-28214, RD12/0042/0016 and PI13/00517, Spanish Gov & FEDER).

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PO415-WED

Direct factor Xa inhibitors paradoxically prolong the half life of active factor Xa in plasma

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Background: Direct FXa inhibitors are anticoagulants that are reversible active-site antagonists of FXa. However, FXa is also normally inhibited by endogenous plasma inhibitors, including antithrombin III (ATIII). ATIII is a suicide substrate that irreversibly inhibits FXa, accounting for the short (~2 min) half-life of active FXa. This is a critical protective mechanism against thrombosis.

Aims: Since inhibition by ATIII requires active site function, direct FXa inhibitors (e.g. rivaroxaban) would interfere with the essential pathway for irreversible protease depletion. Our goal was to address whether rivaroxaban paradoxically prolongs the half life of FXa in plasma by creating a reservoir of reversibly inhibited FXa shielded from irreversible inhibition by ATIII.

Methods: The kinetics of distribution of FXa between different pools was measured in plasma with or without rivaroxaban using an ELISA to track FXa-ATIII complex formation and a biotinylated chloromethylketone (B-EGRCK) to trap and determine the concentration of all FXa not in complex with ATIII. The small amount of free FXa uncomplexed with rivaroxaban or ATIII was interpolated from a thrombin generation assay (TGA).

Results: The presence of rivaroxaban (1 µM) greatly decreased the concentration and rate of FXa-ATIII formation following the addition of FXa. This was associated with a corresponding increase in B-EGRCK labeling, suggesting that FXa not complexed with ATIII is either free or bound to rivaroxaban. In the TGA assay, rivaroxaban paradoxically caused FXa activity to persist for >1 h compared to ~5 min without rivaroxaban.

Conclusion: These results illustrate a previously unreported modulation by rivaroxaban of the critical function of plasma protease inhibitors. Rivaroxaban leads to persistence of FXa in a reversible complex with the inhibitor that is in equilibrium with free FXa. The thrombotic potential of this free FXa is unclear, but it may have important implications for understanding the safety and efficacy of these anticoagulants.

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PO416-WED

Detecting relevant rivaroxaban or dabigatran levels in clinical care by routine coagulation tests or thromboelastography in patients with atrial fibrillation

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Background: In emergency situations, fast and 24/7 available screening tests for detecting anticoagulants are recommended. Traditional coagulation tests are included in emergency guidelines for management of patients on non-vitamin K oral anticoagulants (NOACs) who experience bleeding or require surgery. These tests are not validated in daily care.

Aims: To determine the ability of routine coagulation tests, prothrombin time (PT), thrombin time (TT), partial thromboplastin time (APTT) and ROTEM, to detect levels as low as 30 ng mL⁻¹ dabigatran (dabi) or rivaroxaban (riva) in real-life steady state patients.

Methods: In an observational study, 76 patients (30 dabi, 46 riva) with non-valvular atrial fibrillation were structurally followed-up. 148 blood samples (75 dabi, 109 riva) were collected to determine PT (Innovin, Neoplastin), aPTT (Actin FSL, Cephascreen), TT (Thromboclotin, Thrombin) on CS2100/STA-R and CT-INTEM/EXTEM on ROTEM. The cut-off for detection was set at <30 ng mL⁻¹ for both riva and dabi. As a reference dabi and riva activity was determined by diluted TT Hemoclot and DiXaI test (both Biophen) on CS2100.

Results: APTT Cephascreen, and both TT reagents detected dabi activity > 30 ng mL⁻¹ in all samples. APTT Actin FSL, PT Neoplastin and PT Innovin detected respectively 91%, 92% and 49%. Riva activ-

ity was best detected by PT Neoplastin (97%) and APTT Cephascreen (88%). APTT Actin FSL and PT Innovin only detected 67% and 66%. For logistical reasons, ROTEM was not determined in all samples; CT-EXTEM detected $> 30 \text{ ng mL}^{-1}$ in 96% (25/26) of riva samples, and CT-INTEM detected 86% (18/21) of dabi samples.

Conclusion: In patients with AF, the ability of routine coagulation tests, to detect the presence of significant levels of dabigatran, or rivaroxaban, is extremely test and reagent dependent. Emergency care protocols should ensure that local test reagents are sufficiently accurate for detecting the presence of NOACs in blood samples

Disclosure of Interest: None Declared.

PO417-WED

Correlation of chromogenic rivaroxaban anti-Xa and dabigatran anti-IIa activity with routine prothrombin time and activated partial thromboplastin time

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Background: New oral anticoagulants do not require regular monitoring however they do potentially prolong the Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) depending on reagent sensitivity. Within a busy diagnostic laboratory this frequently presents as unexplained prolonged PT or APTT at screening but also PT and APTT measurement form the first line of investigation particularly outside of 'core hours' for assessment of anticoagulant activity in patients presenting with bleeding complications where specific anti-Xa or IIa assays are not readily available.

Aims: To determine sensitivity of PT and APTT to Rivaroxaban and Dabigatran and establish a 'therapeutic' range for each based on the PT or APTT for estimation of anticoagulant effect in emergency situations.

Methods: Assays were performed on Destiny Max coagulation analyser (Stago UK), PT and APTT measured using mechanical clot detected using TiniCLOT PT, TriniCLOT aPTT reagents with TriniCHECK controls. Dabigatran concentration determined using Biophen DTI chromogenic Anti-IIa assay, using specific calibrators and controls (Hyphen BioMed). Rivaroxaban concentration determined using chromogenic Liquid Anti-Xa assay (Stago UK), using specific calibrators and controls. Correlation was performed using citrated patient plasma samples.

Results: Good correlation was seen between PT and Rivaroxaban concentration $r = 0.92$ and APTT with Dabigatran concentration $r = 0.83$. Peak Rivaroxaban range $175\text{--}360 \text{ ng mL}^{-1}$ corresponds to PT $17.7\text{--}23.5 \text{ s}$ (normal $11.6\text{--}13.9 \text{ s}$). Peak Dabigatran range $64\text{--}443 \text{ ng mL}^{-1}$ corresponds to APTT $41.5\text{--}71.8 \text{ s}$ (normal $24.5\text{--}37.1 \text{ s}$).

Conclusion: PT or APTT showed good correlation with Rivaroxaban or Dabigatran concentration. Both PT and APTT reagents were sensitive and prolonged within respective peak ranges. PT or APTT reagents evaluated can give a good indication of anticoagulant activity of Rivaroxaban or Dabigatran in clinical emergency situations.

Disclosure of Interest: None Declared.

PO418-WED

Four-factor prothrombin complex concentrate (4F-PCC) reverses bleeding associated with new oral anticoagulants (NOAC) dabigatran, rivaroxaban, edoxaban and apixaban in a rabbit model of acute bleeding

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Background: Major bleeding remains a significant risk of the new target specific oral FIIa and FXa inhibitors. Unlike traditional anticoagulants, these newer agents do not have specific, validated anticoagulant reversal strategies, which may be required during emergency surgery, serious bleeding or anticoagulant overdose. The four-factor prothrombin complex concentrate (4F-PCC) Beriplex® P/N has been demonstrated to effectively control acute new oral anticoagulant (NOAC) associated bleeding in an *in vivo* rabbit model.

Aims: This presentation gives a comprehensive overview of the efficacy data obtained using this animal model, including 4F-PCC mediated reversal of the FIIa inhibitor dabigatran, and the FXa inhibitors rivaroxaban, edoxaban and apixaban together with the correlation between *in vivo* measures of haemostasis and *in vitro* coagulation parameters.

Methods: Anesthetized rabbits were treated with a single i.v. dose of dabigatran ($400 \mu\text{g kg}^{-1}$), rivaroxaban ($300 \mu\text{g kg}^{-1}$), edoxaban ($1200 \mu\text{g kg}^{-1}$) or apixaban ($1200 \mu\text{g kg}^{-1}$) followed by 4F-PCC (Beriplex® P/N; $6.25\text{--}100 \text{ IU kg}^{-1}$). Bleeding was induced by a standardized kidney incision and quantified by measurements of volume of blood loss and time to hemostasis. In parallel, blood samples were collected for evaluation of coagulation parameters.

Results: The studies confirmed a dose-dependent increase in time to haemostasis and total blood loss after NOAC administration. Subsequent treatment with clinically relevant doses of 4F-PCC was able to statistically significantly reduce bleeding signals. Of the coagulation parameters measured, thrombin generation (peak and ETP) was the most sensitive to 4F-PCC mediated reversal, correlating best with *in vivo* measure of haemostasis, although correlations were also observed for prothrombin time (PT), whole blood clotting time (WBCT) and thromboelastography parameters (TEG).

Conclusion: Overall, in this rabbit model of haemostasis, 4F-PCC showed potential for reversing the bleeding effects of all NOACs tested.

Disclosure of Interest: E. Herzog Employee of: Employee of CSL Behring GmbH, F. Kaspereit Employee of: Employee of CSL Behring GmbH, W. Kregge Employee of: Employee of CSL Behring GmbH, J. Mueller-Cohrs Employee of: Employee of CSL Behring GmbH, B. Doerr Employee of: Employee of CSL Behring GmbH, P. Niebl Employee of: Employee of CSL Behring GmbH, D. Gerhard Employee of: Employee of CSL Behring GmbH.

PO419-WED

Stability of renal function in patients with atrial fibrillation switched from warfarin to target-specific oral anticoagulants

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Background: Four target-specific oral anticoagulants (TSOACs), dabigatran, rivaroxaban, apixaban, and edoxaban are approved in the United States as alternatives to warfarin for stroke prevention in atrial fibrillation (AF). Unlike warfarin, TSOAC dosing is based primarily on renal function.

Aims: To identify changes in renal function and associated dosing implications in a multicenter cohort of AF patients treated with TSOACs.

Methods: Among six anticoagulation management clinics in the Blue Cross Blue Shield of Michigan sponsored Michigan Anticoagulation Quality Improvement Initiative, 190 AF patients who switched from warfarin to a TSOAC between 2009 and 2014 were followed with institutional review board approval. Patients were grouped based on baseline creatinine clearance (CrCl) estimated using the Cockcroft-Gault equation and their lowest observed renal function during followup (average of 591 days). Compliance with FDA-recommended dosing based on renal function was assessed.

Results: Of the 190 AF patients switched from warfarin to a TSOAC, 147 (77.4%), 35 (18.4%) and 8 (4.2%) had baseline CrCl ≥ 50 , 30–49, and <30 mL min⁻¹, respectively. Of the 106 patients switched from warfarin to dabigatran, 90 (84.9%) had a baseline CrCl ≥ 50 mL min⁻¹, of which none had a CrCl during follow up that would require a dose change. Of the 53 patients switched from warfarin to rivaroxaban, 36 (67.9%) patients had a baseline CrCl ≥ 50 mL min⁻¹, and 2 (5.6%) had a follow up CrCl requiring a dose change. Of the 31 patients switched from warfarin to apixaban, 27 had baseline mean CrCl of 86 mL min⁻¹, and 5 (16.1%) patients had a follow up creatinine requiring a dose change.

Conclusion: Over the follow up period, most AF patients who switched from warfarin to a TSOAC had stable renal function. Few patients required TSOAC dosing changes based on changes in renal function. As the use of TSOACs expands, monitoring of renal function and appropriate dose adjustments are critical.

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PO420-WED

Bleeding on novel oral anticoagulation – our experience in the Northern Region, United Kingdom: presenting features and management of bleeding in individuals treated with a novel oral anticoagulant (NOAC): results of an observational cohort study

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Background: There is increasing use of novel oral anticoagulants (NOACs) in prevention and treatment of venous thromboembolism and stroke prevention in atrial fibrillation (AF). Dabigatran, Rivaroxaban and Apixaban are currently licensed in the United Kingdom but there is no direct antidote available and optimal management in the event of significant bleeding remains uncertain.

Aims: This prospective, observational cohort study aimed to determine severity, site and management of bleeding events in patients on NOACs within the Northern Region of the UK.

Methods: A questionnaire, using a web based survey tool, was sent to haematologists within the region. Data was collected on patient demographics, anticoagulant and dose, site of bleeding, severity according to ISTH criteria, management and outcome.

Results: 40 bleeding events were reported over a 21 month period, March 2013 to February 2015. NOAC prescribed was; Dabigatran, 20 cases (50%), Rivaroxaban, 19 (47.5%) Apixaban, 1 (2.5%). Median age was 72.7 years. The majority (76.9%) were anticoagulated for AF. 62.5% of bleeding events were classed as severe. Site of haemorrhage included 16 upper GI (56.2% on Dabigatran) and 5 intracranial (10%). 60% of patients presented within 12 h of their most recent dose.

90% of bleeding events on Dabigatran were associated with a prolonged PT and APTT and 80% a prolonged Thrombin Time. For Rivaroxaban, PT and APTT were prolonged in 74% and 68%, respectively, at presentation.

Haemostatic therapy was administered in 13: Prothrombin Complex Concentrate, 11; Recombinant factor VIIa, 1; Factor VIII Inhibitor Bypassing Activity (FEIBA), 1. 2 cases on Dabigatran were managed with haemodialysis. There were 3 deaths attributed to haemorrhage; 1 intracranial, 1 GI and 1 haemorrhage due to disseminated intravascular coagulation and NOAC.

Conclusion: Larger studies are needed to establish bleeding rates for each NOAC and to guide definitive management of significant bleeding prior to the availability of a direct reversal agent.

Disclosure of Interest: None Declared.

PO421-WED

Drug use evaluation of rivaroxaban in Tan Tock Seng Hospital: a review of utilization trends among Asian population

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Background: Since the approval of rivaroxaban by Tan Tock Seng Hospital (TTSH) Pharmacy and Therapeutics (P&T) Committee in Y2009, the utilisation rate has increased at least 3-fold. This is the first rivaroxaban utilization review since its approval in Y2009.

Aims: The study aims to assess the appropriateness of rivaroxaban use in TTSH with due consideration to indication, dose and duration.

Methods: This is a retrospective, single center, cohort study.

Patients who were started rivaroxaban from January 2010 through June 2013 were reviewed for the following:

- 1 Indication
- 2 Dose
- 3 Duration (post-TKR/THR DVT/PE prevention indication)

Adverse outcome was reported as number of bleeding episodes while efficacy was represented by number of DVT and/or PE events within 12 months of rivaroxaban initiation.

Patients who were started rivaroxaban by non-TTSH physicians were excluded. For outcome analysis, patients who were lost to follow up were excluded.

All data were accessed via the institution computerized medical records.

Results: Case records of 266 patients were assessed. Out of the 266 patients, 251 patients were screened for bleeding and efficacy outcomes.

Three out of five patients received rivaroxaban for off-label indications.

23 patients (8.6%) received rivaroxaban at doses greater or lower than FDA recommendation.

39 patients (14.7%) received rivaroxaban at duration longer or shorter than FDA recommendation as per post-TKR/THR DVT/PE prevention.

16 (6.4%) major or fatal bleeding events were reported. Out of the 16 reported bleeding events, 2 patients (overall, 0.8%) died. One episode of stroke (0.4%) and DVT (0.4%) was observed within 12 months of rivaroxaban initiation.

Conclusion: The prescribing pattern of rivaroxaban varied among the various physicians in TTSH. Hospital statements offering guidance towards rivaroxaban prescribing can aid to establish a consensus among the physicians for cost-saving and safety purpose.

Disclosure of Interest: None Declared.

PO422-WED

In vitro assessment of direct oral anticoagulant's hemorrhagic or thrombotic risk by fibrin clot nanostructure profiles

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Background: The central clinical concern of the direct oral anticoagulants (DOACs) is to strike the right balance between hemorrhagic and thrombotic risk. Current methods evaluate only anti-thrombin or anti-FXa circulating activities which are not predictive of those risks. So, there is a crucial need to assess the risk/benefit ratio of DOACs for managing anti-thrombotic drug therapy.

Aims: We investigated the *in vitro* hemorrhagic or thrombotic risk of the different molecules of DOACs by determining the temporal evolution of fibrin nanostructure (1,2).

Methods: Normal pool plasmas were spiked with serial dilutions (0–400 ng mL⁻¹) of DOACs (dabigatran, rivaroxaban and apixaban) determined by LCMS/MS. The plasmas incubated by low Tissue Factor concentration and t-PA were triggered by calcium. Real-time measurement of the number of protofibrils was monitored during 30 min at 37°C on a prototype routine instrument(1,2).

Results: Our results show independently that the three molecules have different temporal profiles of fibrin nanostructure. In addition, interestingly, we show that the three DOACs increase the rate of clot lysis. This screening method confirms the different safety/efficacy profile of these three molecules.

Conclusion: Our results show that the temporal profile of fibrin clot nanostructure allows determining the hemorrhagic and thrombotic risk. Indeed, the DOAC molecules have different impacts on the fibrin nanostructure during clotting and fibrinolysis, and hence on the bleeding risks. So, the fibrin clot nanostructure profile could be a major determinant of hemorrhagic and thrombotic risk subtraction. (1) Dassi et al. ISTH 2015 (submitted), (2) Yeromonahos et al. Biophysical J. 2010, Arterioscler Thromb Vasc Bio. 2012.

Disclosure of Interest: None Declared.

PO423-WED

Use of non-vitamin K antagonist oral anticoagulants for non-valvular atrial fibrillation in real-life: comparison with phase III trials

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Background: Whether patients prescribed with non-vitamin K antagonist oral anticoagulants (NOACs) or vitamin K antagonists (VKAs) for non-valvular atrial fibrillation (NVAF) in real-life are comparable to those included in phase III trials is undefined

Aims: To compare patients prescribed with NOACs or receiving VKAs for NVAF with those of patients included in phase III trials and registries

Methods: Consecutive patients with NVAF prescribed with NOACs and a random sample of patients with NVAF receiving VKAs were included in a prospective cohort

Results: Overall, 1494 patients with NVAF were included: 1021 receiving NOACs and 473 VKAs. Apixaban, dabigatran or rivaroxaban were prescribed in 279, 386 and 356 patients, respectively.

CHADS₂ ≥ 3, age ≥ 75 or previous stroke were more common in apixaban or dabigatran prescribed patients than in ARISTOTLE ($P < 0.001$; $P < 0.001$; $P < 0.001$) or RE-LY ($P = 0.0059$; $P < 0.001$; $P < 0.001$) patients. Rivaroxaban prescribed patients less frequently had a CHADS₂ ≥ 3 ($P < 0.001$) or a previous stroke ($P < 0.001$) and more frequently age ≥ 75 ($P < 0.001$) compared to ROCKET patients. Apixaban or rivaroxaban prescribed patients more commonly had HASBLED > 3 respect to ARISTOTLE ($P = 0.044$) or ROCKET ($P < 0.001$) patients.

CHADS₂ ≥ 3, HASBLED > 3 or previous stroke were more common in dabigatran prescribed patients or VKAs patients ($P < 0.001$ for all comparisons) compared to patients included in the Medicare cohort. Compared to the Danish cohort, CHADS₂ ≥ 2 and HASBLED ≥ 2 were more common in dabigatran prescribed patients (for both 150 or 110 dosages) and in VKAs patients ($P < 0.001$ for all comparisons). Previous stroke was more common in dabigatran, rivaroxaban or apixaban prescribed patients compared to the Danish cohort ($P < 0.001$, $P = 0.0016$ and $P < 0.001$, respectively)

Conclusion: Real-life patients prescribed with NOACs are different from study populations included in phase III trials in terms of both thrombotic and haemorrhagic risks. Real-life outcome data are needed to confirm the results of phase III trials

Disclosure of Interest: None Declared.

PO424-WED

Comparison in assessment of the kinetics of NOACS (Dabigatran, Rivaroxaban, Apixaban) between target specific assays and common coagulation tests assaysArtang R¹, Nielsen JD², Dunois C³, Amiral C³ and Amiral J³¹Cardiology, University of Nebraska Medical Center, Omaha, USA; ²Coagulation Laboratory, Copenhagen University Hospital, Bispebjerg, Denmark; ³Research, HYPHEN BioMed, Neuville sur Oise, France

Background: New (Direct) Oral Anti-Coagulants (NOACs) have increasing therapeutic applications, and do not require routine monitoring, excepted in some specific conditions, or before surgery in treated patients. When needed, patient testing must be performed in emergency with available appropriate assays.

Aims: The purpose of this study was to evaluate the anticoagulant kinetics of 3 NOACs (Apixaban, Dabigatran and Rivaroxaban) in healthy volunteers, as measured with various coagulation assays or specific methods; this gives indications on which assays are possible when patient testing is necessary.

Methods: Ten healthy volunteers were given single dose of Dabigatran (150 mg), or Rivaroxaban (20 mg), or Apixaban (10 mg). Blood was collected before intake, or 2, 4 or 24 h after. PT and APTT were performed on all specimen, and Dabigatran samples were also tested with TT, Hemoclot Thrombin Inhibitors (HTI) or Biophen DTI (BDTI chromogenic), whilst Apixaban and Rivaroxaban were tested with 2 anti-Xa assays (kinetics Anti-Xa test for heparin or 2 stage specific chromogenic assay). Results were expressed as mean value +/- SD. Assays were calibrated with dedicated calibrators for each NOAC (established with LC:MS/MS).

Results: For all NOACs the peak concentration was observed between 2 and 4 h (mean ng/ml: 185 +/- 67 for Apixaban, 94 +/- 70 for Dabigatran and 203 +/- 120 for Rivaroxaban), with concentrations all < 50 ng mL⁻¹ at 24 h. PT and APTT offered low sensitivity and no reliability, whilst HTI and BDTI gave quantitative and similar values for Dabigatran as did the 2 anti-Xa assays for Apixaban and Rivaroxaban. TT was very sensitive for low Dabigatran concentrations (< 30 ng mL⁻¹).

Conclusion: Accurate measurements of NOACs can be achieved on plasma, using specific assays and dedicated calibrators, when required. TT is useful for excluding presence of Dabigatran, especially before surgery at bleeding risk. Use of global assays is not recommended for quantitative measurements of NOACs.

Disclosure of Interest: R. ARTANG Speaker Bureau of: Bristol-Meyers Squibb, J. Dalsgaard Nielsen:None Declared, C. Dunois Employee of: HYPHEN BIOMED, C. AMIRAL Employee of: HYPHEN BIOMED, J. AMIRAL Consultant for: HYPHEN BIOMED and Sysmex.

PO425-WED

Anticoagulation pharmacokinetics during dabigatran therapy

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Background: The direct acting anticoagulant dabigatran is relatively short-lived in blood, with free drug concentrations directly proportional to its function.

Aims: To assess dabigatran efficacy computationally and empirically at free drug C_{max} and C_{trough} concentrations measured at the 5th, 50th and 95th pharmacokinetic (PK) percentiles (150 mg bid).

Methods: Clotting times in tissue factor initiated contact pathway inhibited blood ± dabigatran were measured (N = 5 donors). The fold change in clot time induced by each concentration of dabigatran in each individual's blood was calculated relative to a simultaneous control without dabigatran. Computationally derived thrombin genera-

tion profiles were generated using coagulation factor concentrations from 32 healthy individuals ± dabigatran at free drug C_{max} and C_{trough} measured at the 5th, 50th and 95th PK percentiles. Clot times extracted from each profile in the presence of dabigatran divided by the pretreatment value for that individual yield a ratio (cINR), similar to a PT derived INR, with values between 2 and 3 suggesting safe anticoagulation.

Results: cINR and fold prolongation estimates of dabigatran efficacy show good correspondence at concentrations spanning the entire PK range. At median PK between C_{trough} and C_{max}, dabigatran maintains cINR values between 2.1 and 3.1 for all 32 individuals and clot time prolongation values between 1.8 and 2.8. Individuals in the 5th PK percentile are adequately anticoagulated as they approach C_{max} (mean cINR=2, range 1.9-2.1) but under anticoagulated as they approach C_{trough} (mean cINR=1.6, range 1.5-1.7); fold prolongation at C_{trough} ranged from 1.5 to 1.6. In the 95th percentile, all individuals are over anticoagulated with mean cINR values ranging from 3.2 to 4.2 over the 12 h cycle; fold prolongation at C_{max} ranged from 3.3 to 3.7.

Conclusion: Individual differences in PK behavior have a greater impact on achieving adequate anticoagulation than differences due to normal range variation in proteome composition.

Disclosure of Interest: T. Orfeo: None Declared, M. Gissel: None Declared, K. Mann Shareholder of: Haematologic Technologies, Consultant for: Alnylam, Baxter, Bayer, CSL Behring, Diagnostica Stago.

PO426-WED

Efficacy of prothrombin time and activated partial thromboplastin time as a gauge for specific factor inhibitionKeller C¹, Dennis J², Monroe D², Tidwell R² and Church F²¹Biology; ²Pathology, University of North Carolina at Chapel Hill, Chapel Hill, USA

Background: The increased interest and development of oral antithrombotic agents targeted to one specific coagulation factor requires effective assays to determine factor inhibition. Current assays may not accurately detect the presence of these highly targeted antithrombotics.

Aims: The aim was to determine the correlation between PT and aPTT and individual factor inhibition.

Methods: A library of dicationic compounds developed for treatment of trypanosomiasis was screened for coagulation inhibition. The inhibitors were taken up in dimethyl sulfoxide. Individual factor inhibition was determined by chromogenic substrate assays. Purified coagulation factors were incubated with each inhibitor and the amount of substrate cleavage was measured. Prothrombin Time (PT) and activated Partial Thromboplastin Time (aPTT) for each compound via Diagnostica Stago STA 4 hemostasis analyzer, Diagnostica Stago silica activator with 25 mm Diagnostica Stago calcium chloride, Diagnostica Stago anti-heparin calcium thromboplastin, and Affinity Biologicals Visu-Con-F control and Visu-Cal-F calibrator plasmas.

Results: We examined the data of compounds that inhibited FIIa, FVIIa, FIXa, FXa, and/or FXIa for prolongation of PT and aPTT. Inhibition of any individual factor did not correlate with prolonged coagulation. aPTT prolongation was seen with inhibition of three or more factors, most of which included thrombin and Factor Xa. Inhibition of FVIIa, FIXa, or FXIa without FIIa and/or FXa inhibition demonstrated normal PT and aPTT.

Conclusion: Individual factor inhibition showed prolongation of neither PT nor aPTT. The use of these assays is better suited for analysis of multifactor inhibition rather than determination of individual factor inhibition. Our observations are consistent with observations of currently marketed specific factor anticoagulants that are not usefully monitored by PT and aPTT. Based on our data, development of new clinical assay methods should accompany the development of targeted antithrombotic compounds.

Disclosure of Interest: C. Keller: None Declared, J. Dennis Grant/Research Support from: Diagnostica Stago, Inc., D. Monroe Grant/Research Support from: Diagnostica Stago, Inc., R. Tidwell: None Declared, F. Church Grant/Research Support from: Diagnostica Stago, Inc.

PO427-WED

Target specific oral anticoagulants can cause false-positivity of confirmatory lupus anticoagulant testing, especially in DRVVT testing

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Background: Target specific oral anticoagulants (TSOACs) are widely used for prevention and treatment of thrombosis. However, little information is available on TSOACs and interference with lupus anticoagulant (LA) testing.

Aims: The aim of this study was to describe the false-positive rates of LA tests in patients receiving TSOACs.

Methods: 337 patients received rivaroxavan (RIV) and dabigatran (DAB) were enrolled. Among them, 35 patients with previously negative LA test results were analyzed. 32 patients were treated with RIV 15, 20, or 30 mg day⁻¹, and 3 patients were treated with DAB 300 mg day⁻¹. Diagnoses were deep vein thrombosis ($n = 19$), pulmonary thromboembolism ($n = 15$), and cardiac arrhythmia ($n = 1$). Prothrombin time (PT) and activated partial thromboplastin time (APTT) were tested. For LA testing, dilute Russell's viper venom time (DRVVT) and silica clotting time (SCT) were evaluated.

Results: Of the patients treated with RIV ($n = 32$), 31.3% had prolonged PT INR and 18.8% had prolonged APTT. Positive DRVVT screening and confirmatory test results were detected in 19 patients (59.4%; median DRVVT screening time, 42.4 s) and 14 patients (43.8%; median DRVVT normalized ratio, 1.35), respectively. In comparison, positive SCT screening test results were detected in 10 patients (31.2%; median SCT screening time, 40.75 s) and all patients showed negative SCT confirmatory test results. The patients treated with DAB ($n = 3$) had 100% normal PT and APTT results. All 3 patients had positive DRVVT screening test results but negative DRVVT confirmatory test results, and 2 of the 3 patients had positive SCT screening test results but negative SCT confirmatory test results.

Conclusion: RIV and DAB affected not only LA screening but also LA confirmatory tests. DRVVT screening and confirmatory tests were more affected than SCT tests. We concluded that SCT-based LA testing is more useful than DRVVT-based LA testing in patients treated with TSOACs.

Disclosure of Interest: None Declared.

Platelet activation/adhesion/aggregation III

PO428-WED

Intravital three-dimensional visualization and quantification of thrombus formation based on non-linear microscope

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Background: The detailed molecular mechanisms of cardiovascular events remains unclear, largely because of an inability to directly visu-

alize and evaluate thrombus formation in living body in single cell levels.

Aims: To elucidate platelets behavior and multi-cellular processes during thrombus formation, we improved *in vivo* imaging technique based on multi-photon microscopy and light-manipulation technique, and assessed thrombus formation processes using three animal models.

Methods: We combined *in vivo* multi-photon microscope technique and light-manipulations system, and induced thrombotic processes. We developed custom software aiming for objective evaluation with high reproducibility, and quantified three-dimensional thrombus volume changes and leukocyte recruitment steps after inductions of thrombus formation.

Results: First, we induced rapidly developing thrombi composed of discoid platelets, which was triggered by ROS production from photochemical reactions. Using this model, we visualized iPS derived artificial platelet kinetics, and elucidated the functional properties in mice and large-sized animals. We also revealed the different aspects of 'newly produced' larger platelets compared from previously circulating 'older' and aged platelets from platelet activation, aggregations, and coagulation processes.

In the second model, thrombus formation was induced by endothelial cell disruption by laser irradiations. With the rapid recruitment of inflammatory leukocytes into damaged area, fibrin net formation and tissue regenerative changes were also observed. TLR4 signaling contributed to these steps, and pretreatment of LPS markedly enhanced inflammatory reactions.

In last model, spontaneous platelet aggregations were induced by transient ischemia and reperfusion. Ly6G+ Ly6C+ CD11b+ cells recruited from bone marrow to ischemic areas.

Conclusion: In sum, the endothelial cell damage levels determined the thrombotic processes, and our imaging system can evaluate the therapeutic strategies against them.

Disclosure of Interest: None Declared.

PO429-WED

C-CBL regulates GPVI-induced thromboxane A₂ generation independently of the Syk-PLC gamma 2 pathway through Y737 phosphorylation

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Background: Collagen is an important platelet agonist that activates Syk-PLCγ2 through the GPVI receptor. c-Cbl negatively regulates GPVI signaling events and is phosphorylated on tyrosines 700, 731, and 774 through Src family kinases and Syk.

Aims: The contribution of phosphorylated tyrosine 731 on c-cbl to GPVI-mediated functional responses has not been elucidated.

Methods: In this study, we focused on the role of Y731 on platelet physiological responses by utilizing c-Cbl(Y737F) platelets from knock-in mice. We measured platelet aggregation, dense granule secretion, Thromboxane A₂ generation, FeCl₂ injury and signaling. We compared the results of c-Cbl(Y737F) platelets with WT platelets.

Results: Aggregation of c-Cbl(Y737F) platelets was delayed at low to intermediate concentrations of convulxin. The effect was more pronounced when low levels of CRP or collagen were employed. ATP secretion by GPVI agonists was also impaired. In addition, GPVI-mediated thromboxane A₂ generation was decreased with a rightward

shift with increasing concentrations of GPVI agonists. There was also an increased time to occlusion in c-Cbl(Y737F) mice compared to WT in the FeCl₃ injury model. GPVI-induced phosphorylation of Syk or PLCg2, and intracellular calcium mobilization, were unaffected in c-Cbl(Y737F) knock-in murine platelets. There was no difference in GPVI-induced phosphorylation of Akt(Ser473) between c-Cbl(Y737F) knock-in and wild-type platelets upon stimulation with GPVI agonists as well as no difference in GPVI-induced Akt phosphorylation in platelets lacking c-Cbl, suggesting that phosphorylated c-Cbl(Y737) is not required for PI3-kinase activation in murine platelets.

Conclusion: The inhibition of platelet physiological responses seen in GPVI-activated c-Cbl(Y737F) platelets appears to be due to a decrease in thromboxane generation. c-Cbl regulates thromboxane generation independently of the Syk-PLCg2 pathway through its interactions with signaling molecules, other than PI3-kinase, binding to phosphorylated Y731

Disclosure of Interest: None Declared.

PO430-WED

Thrombin-stimulated phosphorylation of disabled-2 regulates its interaction with CIN85 during human platelet activation

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Background: Disabled-2 (Dab2) is a platelet protein that regulates haemostasis and thrombosis by functional interplay with G_{12/13}-mediated thrombin signaling. Although Dab2 is known as a phosphoprotein, the function and regulation of Dab2 phosphorylation in platelets are not clear.

Aims: The nature and significance of Dab2 phosphorylation during human platelet activation were investigated.

Methods: Dab2 was immunoprecipitated from the resting and thrombin-stimulated platelets for differential labeling of ¹³CH₂¹³CH₃ and ¹²CH₂¹²CH₃, respectively. After enrichment of the phosphopeptides, the phosphorylation sites were analyzed by LTQ orbitrap and the corresponding phospho-specific antibodies were generated. The protein kinases responsible for and the functional significance of Dab2 phosphorylation were defined by the use of signaling pathway inhibitors/activators, protein kinase assay, and various molecular approaches.

Results: S723 was identified as the Dab2 phosphorylation site induced by the platelet agonists of thrombin, PAR1 and PAR4 peptides, collagen, ADP and U46619. Thrombin-stimulated S723 phosphorylation (pS723) was suppressed by U73122 (PLC inhibitor) and staurosporine (PKC inhibitor) but not by dorsomorphin (AMPK inhibitor) and Y27632 (ROCK inhibitor). Among the protein kinases predicted to phosphorylate S723, ROCKII was the most potent protein kinase for pS723 in vitro. Both PLC and Rho signaling pathways are thereby important to mediate thrombin-stimulated pS723. Notably, a motif adjacent to S723 mediated the binding of Cbl-interacting protein of 85 kDa (CIN85), a regulator of integrin signaling, to Dab2; the binding only occurred when S723 was unphosphorylated. We further demonstrated that platelet activation by thrombin caused an increase in Dab2-pS723 that is likely to regulate platelet signaling by attenuating Dab2-CIN85 interaction.

Conclusion: Phosphorylation of Dab2 at S723 is involved in regulating Dab2-CIN85 binding during platelet activation that shed new insight in platelet biology.

Disclosure of Interest: None Declared.

PO431-WED

Integrin alpha IIb beta 3-mediated C-Src activation: differential binding to inactive and active C-Src

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Background: αIIbβ3-mediated outside-in signaling is initiated by activation of c-Src bound via its SH3 domain to the C-terminal RGT sequence of the β3 cytoplasmic tail (CT). However, it likely that binding would be precluded in inactive c-Src because the linker between the SH2 and kinase domains occludes the β3 binding site.

Aims: To re-examine the interaction between c-Src and β3.

Methods: We used immunoprecipitation to measure β3 binding to c-Src and biophysical techniques to characterize the interaction of the SH3 domain with the β3 CT.

Results: In resting platelets, there was little interaction between c-Src and β3. Following platelet stimulation, c-Src binding to β3 was detected at 10 sec. Phosphorylation of c-Src residue Tyr419, an indicator of c-Src activation, was detected at 20 s. Using SPR spectroscopy to measure the affinity of the C-terminal β3 peptide NITYRGT to the immobilized SH3 domain, we found a K_d > 300 mM. Repeating these measurements by flowing the SH3 domain over the immobilized β3 CT decreased the K_d ≈ 10 μM. We then used NMR to measure the interaction of NITYRGT with the ¹⁵N-labeled SH3 domain. Because NITYRGT binding to the SH3 domain is weak, we used chemical shift perturbations (CSP) to identify the SH3 residues interacting with NITYRGT. NITYRGT interacted with the SH3 domain RT-loop and surrounding residues. A mutant peptide in which RGT was replaced with the β1 residues EGK induced small CSP on the opposite SH3 domain face. To mimic inactive c-Src, we found that the polyproline peptide RPLPLP prevented NITYRGT binding to the RT-loop.

Conclusion: Platelet stimulation induces rapid c-Src binding to the β3 subunit of αIIbβ3 accompanied by c-Src activation. Interaction of active c-Src with β3 occurs at a site overlapping the polyproline binding site in the c-Src SH3 domain and is likely a 2-dimensional ternary interaction where the membrane surface makes an important contribution. By contrast, interactions of inactive c-Src with β3 are weak and insensitive to β3 CT mutations.

Disclosure of Interest: None Declared.

PO432-WED

Orally administered tetrahydrobiopterin (BH4) suppresses platelet aggregation via intraplatelet production of nitric oxide

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Background: Tetrahydrobiopterin (BH4), known co-factor for catecholamines, serotonin and nitric oxide (NO) biosynthesis, is expected to play some roles in thrombogenesis via modulation of platelet agonists or antagonists such as adrenaline, serotonin or NO.

Aims: We evaluated the effect of BH4 on platelet aggregation by single platelet counting method.

Methods: Citrated blood was collected from male ICR mice (6 months old) under pentobarbital anesthesia (80 mg/kg-i.p.) by venipuncture, after oral administration of aspirin (100 mg kg⁻¹), sodium nitropruside (SNP; 50 mg kg⁻¹) or BH4 (10 mg kg⁻¹): (1) Plasma BH4 was

quantified using reversed-phase ion-pair liquid-chromatography with fluorimetric detection by post-column sodium nitrite oxidation. (2) Whole blood samples were stimulated with collagen ($3 \mu\text{g ml}^{-1}$), fixed with 1% formaldehyde, the number of singlet platelet was counted and expressed as platelet aggregation ratio. (3) Plasma was de-proteinized and NO metabolites (NOX) amount was measured with NO_2/NO_3 Assay Kit-FX (Fluorometric). (4) Blood mixed with 1 mM IBMX for 30 min was centrifuged, purified by adding 6% trichloroacetic acid, lyophilized and cGMP was quantified using an enzyme immunoassay kit (Amersham).

Results: (1) Saline control revealed $169.6 \pm 33.1 \text{ nM}$ of BH4 in plasma, and BH4 oral administration increased that to 642.7 ± 220.6 . (2) Orally administered BH4 inhibited platelet aggregation induced by collagen from $58.0 \pm 4.1\%$ of saline-control to $34.6 \pm 5.6\%$ (SNP used as NO-donor resulted $45.2 \pm 10.3\%$). (3) Saline-control detected $2.68 \pm 0.24 \mu\text{M}$ of blood NOX, and SNP increased it up to $5.94 \pm 0.64 \mu\text{M}$, while BH4 showed no significant change ($2.72 \pm 0.19 \mu\text{M}$). (4) Intraplatelet cGMP concentration of saline control ($2.84 \pm 0.42 \text{ pmol}/10^8$ platelets) was increased to 3.88 ± 0.14 by SNP, and to 3.98 ± 0.57 by BH4, respectively.

Conclusion: These results indicate that BH4 may suppress platelet activation by enhancing local NO production within platelet.

Disclosure of Interest: None Declared.

PO433-WED

Drospirenone enhances GPIIb-IX-V mediated platelet activation

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Background: Epidemiological studies recently revealed that using drospirenone (DRSP)-containing contraceptives is associated with increased thrombosis risk in women. But the underlying causality is unclear.

Aims: To study the effects and probable mechanisms of DRSP on coagulation in vitro.

Methods: The effects of DRSP on the activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT) and fibrinogen (FIB) were first measured. Then the effects of DRSP on platelet activation were investigated in response to low levels of collagen, ADP, thrombin, U46619, adrenaline and botrocetin/Von Willebrand factor (VWF).

Results: DRSP has no direct effect on APTT, PT, TT, FIB, and platelet aggregation induced by low levels of collagen, ADP, thrombin, U46619, or adrenaline. Whereas DRSP enhances botrocetin/VWF induced platelet aggregation and VWF receptor glycoprotein Ib-IX-V (GPIIb-IX-V) mediated signaling. This enhancement can be blocked by a progesterone receptor membrane component 1 (PGRMC1) inhibitor AG205, or ADP scavenger apyrase and cyclooxygenase inhibitor indomethacin. Further mechanism analysis indicated that DRSP treatment significantly promoted the 14-3-3 proteins binding to GPIIb, which may result in the enhancement of platelet activation.

Conclusion: Although DRSP did not directly induce platelet activation, it obviously facilitated VWF receptor GPIIb-IX-V-mediated platelet activation probably via enhancing GPIIb-IX association with 14-3-3 proteins. DRSP's potential binding protein PGRMC1 may play roles in this process. Our study also suggested that inhibition of the production of thromboxane A2 and activation of ADP receptors might prevent the side effects of DRSP.

Disclosure of Interest: None Declared.

PO434-WED

A paradoxical haemorrhagic state caused by deletion of the SRC kinase inhibitor CSK is partially rescued by deletion of CD148

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Background: Platelet tyrosine kinase-linked receptors must be tightly regulated in order to prevent bleeding and thrombosis from occurring. However, the mechanism that sets the threshold of signaling from these receptors remains undefined.

Aims: To determine how the Src family kinase (SFK) inhibitor C-terminal Src kinase (Csk) works in conjunction with the receptor-like protein-tyrosine phosphatase (PTP) CD148 to optimize SFK activity and set the threshold of platelet activation.

Methods: Csk and CD148 conditional knockout (KO) mouse models were generated by crossing Csk- and CD148-floxed mice with PF4-Cre transgenic mice. Platelet count and function were measured using standard assays.

Results: Deletion of Csk in the megakaryocyte lineage resulted in severe macrothrombocytopenia, down-regulation of the ITAM-containing collagen receptor complex GPVI-FcR γ -chain and a bleeding diathesis. Csk-deficient platelets did not respond to GPVI agonists, despite containing increased SFK activity; however, they hyper-responded to antibody-mediated cross-linking of the hemi-ITAM-containing podoplanin receptor CLEC-2. Integrin $\alpha\text{IIb}\beta 3$ -mediated platelet spreading was also increased in the absence of Csk. Deletion of both Csk and CD148 rescued platelet count and volume, but failed to rescue GPVI-FcR γ -chain expression. Phosphorylation of the C-terminal inhibitory tyrosine residue of Lyn was abolished in the absence of Csk, and significantly reduced in Fyn and Src. Deletion of both Csk and CD148 marginally rescued phosphorylation of the inhibitory site of all three SFKs, and increased tyrosine phosphorylation of the activation loop.

Conclusion: A lack of a major SFK inhibitor Csk in platelets results in a paradoxical haemorrhagic state due to reduced platelet count and down-regulation of the GPVI-FcR γ -chain complex. Moreover, it establishes Csk-CD148 as a critical regulator of platelet count and the threshold of platelet activation.

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Disclosure of Interest: None Declared.

PO435-WED

Effect of homocysteine and folic acid in Ca^{2+} homeostasis and ROS production in platelets from bile-duct ligation rats

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Background: In previous studies, we found that intracellular Ca^{2+} homeostasis ($[\text{Ca}^{2+}]_i$) was altered in platelets of an experimental model of cholestasis. Several studies indicate that cholestatic diseases are associated with hyperhomocysteinemia and alterations in platelet function. We have hypothesized that the deficiency in acid folic found in rats with cholestasis may explain the hyperhomocysteinemia and the alterations/changes in platelet function, since folic acid plays a role in the regulation of the metabolism of homocysteine (Hcy) and also in ROS production in platelets.

Aims: We investigated the effects of acute Homocysteine and chronic folic acid treatment on platelet aggregation, $[Ca^{2+}]_i$ homeostasis and ROS production.

Methods: Cirrhosis was induced in rats by bile-duct ligation (BDL) and sham rats were used as control. The experiments were performed after 21 days (without ascitis) after surgery. Platelet rich plasma aggregation in response to ADP was analyzed using a lumiaggregometer. To analyze the $[Ca^{2+}]_i$ and ROS by fluorescence spectroscopy, platelet were incubated with fura2 and CM-H₂DCFDA acetyl ester, respectively, and stimulated with thrombin, ADP and Homocysteine. In addition, Some animals were treated with folic acid in drinking water.

Results: In BDL and control, acute treatment with Hcy increased platelet aggregation, $[Ca^{2+}]_i$ and ROS production. Chronic treatment with folic acid decreased aggregation response by 12% in control rats platelets and 50% in BDL and also reduced the production of ROS in response to thrombin, eliminating differences between platelet control and cirrhotic animals.

Conclusion: BDL animals without ascites had an increased platelet aggregation, which is related to an increased $[Ca^{2+}]_i$ mobilization and ROS production. Chronic treatment with folic acid reversed platelet hyperaggregation and normalized the production of ROS and decreased release of Ca^{2+} from intracellular stores.

Disclosure of Interest: None Declared.

PO436-WED

Computational model of microvascular thrombus formation: the impact of damaged area size on thrombus dynamics

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Background: The regulation of thrombus growth and stability is crucial for normal hemostasis, though the mechanisms involved in limitation of thrombus growth are still a matter of controversy. It is generally accepted that ferric chloride-induced thrombi are normally occlusive while laser and micropuncture-induced thrombi do not significantly reduce the blood flow. Recent *in vivo* studies of laser-induced thrombosis in mice revealed core-and-shell architecture of the forming hemostatic thrombus. Dynamics of thrombus shell formed by reversibly adherent discoid platelets determines its size and shape during initial stages of thrombus formation.

Aims: This study aimed at computational analysis of impact that vessel injury size makes on dynamics of reversibly adherent platelets. We proposed that damage length might be crucial parameter determining the outcome of the vessel injury.

Methods: We developed a 2d computational model of platelet plug formation under high-shear conditions corresponding to arterioles and venules. Platelets were considered as semi-rigid discoid particles placed in Newtonian fluid described with Navier-Stokes equations. Constant pressure drop boundary conditions were implemented in order to resolve vessel occlusion. Using quasi-steady flow approximation made it possible to study simulations corresponding to physiologically relevant timescales of dozens of seconds.

Results: In case of small-scale endothelium damage of several microns our model demonstrates unstable behavior of thrombus shell which is consistent with recent *in vivo* observations. Larger-scale damage of 40 microns results in larger stable thrombus leading to full occlusion of 35 μ m – wide vessel.

Conclusion: Damage length along the blood vessel might be important factor determining the size and stability of the platelet plug formed under high shear conditions. These results might explain the differences of vessel injury outcomes observed for various thrombosis models.

Disclosure of Interest: None Declared.

PO437-WED

Sphingosine kinase deficiency modulates platelet activation

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Background: Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite that plays key roles in vascular development and inflammation. Sphingosine kinases (Sphks) 1 & 2 are together responsible for phosphorylation of sphingosine to S1P in mice. Platelets are an abundant source of S1P, some of which is released upon platelet activation and blood coagulation.

Aims: We wished to evaluate the role of platelet-derived S1P in the modulation of platelet functions *in vivo* and *in vitro* using mice deficient in Sphks 1 & 2 in megakaryocytes/platelets.

Methods: *Sphk1^{fl/-};2^{fl/-};PF4Cre⁺* (platelet S1P-less) mice were generated using a PF4-Cre allele and compared to *Sphk1^{fl/-};2^{fl/-};PF4Cre⁻* littermates (control). *In vitro* assays included S1P quantification in serum, plasma and supernatants of activated platelets by LC-MS/MS, washed platelet aggregation, calcium mobilization, spreading on fibrinogen and P-selectin expression. *In vivo* assays were bleeding time (tail cut) and carotid arterial thrombosis (15% FeCl₃ patch).

Results: While levels of S1P in plasma were unchanged by platelet Sphk deficiency, they were substantially decreased in serum and in supernatant of activated washed platelets. Collagen- and ADP-induced platelet aggregation was slightly impaired, P-Selectin expression in response to thrombin receptor activation delayed, and lamellipodia formation on fibrinogen significantly reduced in washed S1P-less platelets relative to littermate controls. However, platelet Sphk/S1P deficiency did not influence tail bleeding times or time to ferric chloride-induced occlusion of the carotid artery.

Conclusion: Specific deficiency in Sphks in platelets blunted platelet activation and reduced platelet spreading on fibrinogen *in vitro*, but did not significantly influence hemostasis or thrombosis in models tested. While this suggests that S1P modulates platelet function, it also indicates that platelet S1P deficiency can be compensated for by other S1P sources *in vivo*.

Disclosure of Interest: None Declared.

PO438-WED

Molecular characterization of gap junction mediated intercellular communication in platelets

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Background: Although platelets circulate in isolation, upon activation they accumulate or aggregate together to form a thrombus, where they function in a coordinated manner to prevent bleeding and control wound repair. It has been reported that the stability and functions of a thrombus are maintained through sustained, contact dependent signalling between platelets through various membrane proteins. Recently, we explored the presence of multiple connexins in platelets and demonstrated the roles for Cx37 and Cx40 in the regulation of platelet function using pharmacological agents that block these molecules and Cx-deficient mice.

Aims: To establish a protein microarray technology in order to characterize platelet intercellular communication and the molecules that are transported between platelets through gap junction channels.

Methods: A microarray of uniformly distributed spots of Alexa Fluor⁶⁴⁷ labelled fibrinogen was developed on glass slides and this enabled the organized and controlled spreading of human washed platelets. The cells were processed either in live or fixed conditions for further imaging using ultrafast/super resolution confocal microscopy.

Results: This protein microarray technique enabled the formation of a lawn of platelets which touch each other on the fibrinogen-coated surface and allowed us to monitor the synchronized signalling events in platelets such as calcium mobilization. Development of this microarray allowed the precise characterization of intercellular communication between platelets using confocal microscopy. We are further developing this technique to understand the detailed mechanisms through which platelet hemichannels and gap junction function are regulated in platelets.

Conclusion: Together, these data provide a platform for detailed characterization of connexin functions with implications for the development of improved anti-platelet therapies.

Disclosure of Interest: None Declared.

PO439-WED

Impaired platelet responses to thromboxane A₂ and thrombin in mice lacking receptor-interacting protein kinase 3

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Background: Receptor-interacting protein kinase 3 (RIP3) is vital for regulating necroptosis, but its role in thrombosis and hemostasis remains unclear.

Aims: To investigate the role of RIP3 in thrombosis and hemostasis.

Methods: Platelet aggregation and secretion were recorded at real time. Platelet P-selectin exposure and specific fibrinogen binding were detected by flow cytometry. TXA₂ generation was measured with enzyme immunoassay (EIA) kit. Protein phosphorylations were detected by western blotting.

Results: Here we show that RIP3^{-/-} mice had tail-bleeding times that were significantly prolonged compared with their wild-type littermates. In an *in vivo* model of mesenteric arteriole thrombosis, mice lacking RIP3 exhibited prolonged occlusion times. Moreover, we found that RIP3 was expressed in platelets. Wild-type mice repopulated with RIP3^{-/-} bone marrow-derived cells had longer occlusion times than RIP3^{-/-} mice repopulated with wild-type bone marrow-derived cells. Deletion of RIP3 in mouse platelets caused a marked defect in aggregation and attenuated dense granule secretion in response to low doses of thrombin or a thromboxane A₂ (TXA₂) analogue, U46619, and addition of exogenous ADP rescued the reduced platelet aggregation. Although TXA₂ generation and α-granule secretion were not impaired, integrin α_{IIb}β₃ activation was attenuated in RIP3^{-/-} platelets. Finally, we found phosphorylation of Akt induced by U46619 or thrombin was diminished in platelets lacking RIP3, and activation of Akt signalling restored the impaired aggregation of RIP3^{-/-} platelets. These data demonstrate a novel role for RIP3 in amplifying U46619- and thrombin-induced platelet activation by mediating Akt-dependent ADP secretion, and in supporting hemostasis and thrombus formation *in vivo*.

Conclusion: These data demonstrate a novel role for RIP3 in amplifying U46619- and thrombin-induced platelet activation by mediating Akt-dependent ADP secretion, and in supporting hemostasis and thrombus formation *in vivo*.

Disclosure of Interest: None Declared.

PO440-WED

Lysine acetyltransferase supports platelet function

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Background: The reversible acetylation of protein lysine ε-amino groups, catalyzed by lysine acetyltransferases and deacetylases, serves as a molecular switch in the orchestration of diverse cellular activities.

Aims: Here, we aim to describe a role for lysine acetyltransferase in platelet activation and hemostatic function.

Methods: Proteomic, biochemical, super resolution microscopy and other cell biological methods were used to study the platelet acetyllysine proteome and acetyltransferase enzymes. *In vitro* analyses of platelet aggregation and platelet spreading as well as *ex vivo* assays of platelet aggregation in whole blood under shear were used to examine platelet hemostatic function.

Results: We identified 541 acetyllysine modifications on 289 platelet proteins that serve metabolic, cytoskeletal, signaling and other cellular functions. Platelets express the lysine acetyltransferase p300, which is activated in a Src kinase-dependent manner upon platelet stimulation with the platelet glycoprotein VI (GPVI) agonist CRP. Inhibition of platelet p300 abrogated CRP-driven actin, filamin and cortactin acetylation as well as F-actin polymerization and platelet aggregation. Super resolution visualization of platelet actin-rich adhesion structures revealed abundant acetyllysine protein content colocalized with actin cytoskeletal proteins. Inhibition of p300 blocked platelet filopodia formation and the spreading of platelets on fibrinogen and collagen surfaces. In whole blood, p300 inhibition prevented the formation of platelet aggregates under shear, suggesting a physiological role for lysine acetyltransferase activity in platelet function.

Conclusion: Together, our findings uncover lysine acetyltransferase as a potential regulator of platelet activation and reveal potential roles for lysine acetylation in the molecular coordination of platelet function. Accordingly, lysine acetyltransferase enzymes and effectors may provide novel targets for the detection, management and treatment of platelet-based disease states.

Disclosure of Interest: None Declared.

PO441-WED

Glycoprotein VI (GPVI) is internalized in platelets activated by soluble GPVI-specific agonists

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Background: Internalization of membrane receptors upon ligand engagement may serve to down-regulate their function or transport signals from the periphery to the cell interior. Expression of the platelet collagen receptor glycoprotein VI (GPVI) dramatically decreases in platelets induced by soluble GPVI-specific agonists, so a similar phenomenon is occurring with GPVI.

Aims: To determine if loss of surface GPVI in platelets activated by GPVI-specific agonists is due to internalization and its possible mechanism.

Methods: Surface GPVI loss was determined in fluorescently labeled 204-11 Fab (GPVI-dimer-specific) or 1G5 (pan GPVI antibody) labeled platelets by flow cytometry and imaging by confocal microscopy.

Results: (1) Reduction of platelet surface GPVI expression is only induced by GPVI-specific agonists (CRP, Cvx). (2) Rapid surface

GPVI loss (<2 min) due to internalization, not shedding. (3) In platelets induced by CRP or Cvx, GPVI was internalized to the cell center (confocal microscopy). (4) Sulfo-NHS-SS-biotin-1G5 treated with excess Alexa-Fluor-647-streptavidin was reacted with platelets. Confocal microscopy indicated that Cvx- or CRP-activated platelets contained internalized GPVI; treatment with the membrane-impermeant reducing agent TCEP [tris(2-carboxyethyl)phosphine hydrochloride] did not reduce this internalized fluorescence, but reduced the surface fluorescence of both resting and thrombin-activated platelets, supporting GPVI internalization. (5) Actin antagonists prevented GPVI internalization, suggesting cytoskeletal protein involvement.

Conclusion: We previously demonstrated GPVI-dimer-clustering on the surface of platelets adhered to immobilized collagen. In contrast, platelet binding to a soluble GPVI-ligand results in internalization of GPVI-ligand complexes. Since actin antagonists inhibit both GPVI clustering and internalization, we will be determining if both processes may in part involve similar mechanisms.

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Disclosure of Interest: None Declared.

PO442-WED

Release of extra cellular DNA contributes to renal ischemia reperfusion injury through platelet activation and formation of neutrophil extracellular traps

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Background: Renal ischemia reperfusion injury (I/R) results from a complex interplay between coagulation and inflammation, resulting in tissue damage. It has been shown that platelet inhibition protects from I/R. How platelets are activated upon renal I/R is not entirely known.

Aims: To investigate renal cell death, extracellular DNA release and neutrophil extracellular traps (NETs) as potential triggers for platelet activation and renal I/R injury.

Methods: We stimulated platelets with necrotic renal cells in presence or absence of deoxyribonuclease 1 (DNase1). Platelet activation and platelet-leukocyte formation were measured by FACS. We stimulated granulocytes with activated platelets and measured DNA release – indicative of NET formation. Vice versa we stimulated platelets with NETs and measured PF4 release. Mice were subjected to renal I/R and treated with DNase1 or vehicle, and sacrificed after 1 day. As marker for NET formation in tissue we stained renal tissue for citrullinated histone H3 and granulocyte marker Ly6G. In blood we measured Platelet Factor 4 (PF4), DNA, creatinine and urea.

Results: Supernatant from necrotic renal cells stimulated platelet activation and platelet-leukocyte complex formation. Incubation with DNase1 reduced platelet activation. Activated platelets generated NETs in vitro and, vice versa, NETs stimulated platelet activation in vitro. Mice subjected to renal I/R showed a significant increase of extracellular DNA and PF4 levels in the circulation. Treatment with DNase1 lowered DNA and PF4 and improved renal function upon I/R. Immunostainings revealed presence of NETs in renal tissue after I/R. DNase1 treatment resulted in a trend towards fewer NETs and granulocytes in tissue.

Conclusion: Both DNA from necrotic kidney cells and NETs activate platelets, which in their turn cause further NET formation, leading to a vicious triad in the pathogenesis of I/R injury. Treatment with DNase1 may have therapeutic benefits in the context of renal I/R injury.

Disclosure of Interest: None Declared.

PO443-WED

Tractopods: novel integrin alpha(IIB) beta(3) dependent platelet membrane anchors promoting platelet-endothelial interactions and thrombo-inflammation

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Background: Platelets have an important proinflammatory function linked to a broad range of human diseases, including ischemia-reperfusion (I/R) injury. However, the mechanisms by which platelets adhere to the endothelium to promote leukocyte recruitment to the ischemic microvasculature remains ill-defined.

Aims: To investigate the mechanisms underpinning platelet mediated leukocyte recruitment to sites of endothelial inflammation.

Methods: Mouse models of endothelial inflammation in conjunction with a novel high-resolution intravital imaging system and multimode total internal reflection microscopy (TIRF) were utilized to investigate platelet and leukocyte responses in vitro and *in vivo*.

Results: Here we demonstrate the existence of a novel platelet adhesion structure that we have termed tractopods (Latin: *tractus* – to pull; Greek; *pod* – foot; tractopod = pulling foot). Tractopods mediate the stable interaction of principally discoid platelets to sites of endothelial inflammation. In contrast to previously described filamentous tethers, tractopods exhibit a complex, branched morphology induced by localized cytoskeletal remodeling. Tractopod formation is initiated by integrin $\alpha_{IIb}\beta_3$ by outside-in signaling events that are sufficient to promote stable platelet adhesion and low level P-selectin expression, independent of thrombin, adenosine diphosphate (ADP) and thromboxane A₂ (TXA₂). Tractopod-adherent platelets mediate the slow, progressive accumulation of leukocytes to sites of endothelial perturbation over several hours and thus serve to promote thrombo-inflammatory conditions, such as I/R injury.

Conclusion: These studies provide new insights into the mechanisms by which platelets generate sustained adhesion contacts with the inflamed endothelium *in vivo* and defines a novel platelet adhesion structure that plays a central role in mediating leukocyte recruitment to sites of vascular inflammation.

Disclosure of Interest: None Declared.

PO444-WED

Specific regulation of mouse platelet ADP secretion by sarco-endoplasmic reticulum calcium atpase type 3

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Background: Sarco-Endoplasmic Reticulum Ca²⁺ ATPases (SERCAs) are central to platelet Ca²⁺ homeostasis: they maintain both low cytosolic [Ca²⁺], preventing unsolicited platelet activation and high concentration levels in Ca²⁺ stores, a pre-requisite to stimulus-dependent Ca²⁺ mobilisation, and modulation of Ca²⁺ signals. However a direct link between SERCA activity and platelet functions has never been established.

Aims: We have decided to look for a potential role of SERCA3 in platelet functions.

Methods: The haemostasis status of SERCA3^{-/-} mice was characterized and their platelets were studied for adhesion, aggregation, secretion as well as Ca²⁺ mobilisation and PKC phosphorylation.

Results: Here, we show that SERCA3^{-/-} mice exhibit prolonged tail bleeding time and re-bleeding, as well as delayed thrombus formation

in both arteries and venules in an *in vivo* ferric chloride-induced thrombosis model. The involvement of platelets was confirmed *in vitro* by i) defective adhesion and thrombus growth over collagen, in high and low shear conditions, ii) weaker aggregation and dense granule secretion induced by low concentration of collagen or thrombin, iii) lower PKC activity and Ca^{2+} mobilisation in response to thrombin or PAR4-AP.

Most importantly, ADP removal by apyrase diminished adhesion, thrombus growth, PKC activity and Ca^{2+} mobilization of control platelets to the level of SERCA3^{-/-} platelets. Altogether these results demonstrate that SERCA3 ablation leads to low ADP secretion in mouse platelets. Interestingly, differential ADP secretion between control and SERCA3^{-/-} platelets was unaffected by $\alpha_{\text{IIb}}\beta_3$ integrin blockade, suggesting a defect independent from $\alpha_{\text{IIb}}\beta_3$. Accordingly, outside-in signalling was unaffected since spreading on fibrinogen, clot retraction and $\alpha_{\text{IIb}}\beta_3$ -dependent dense granule secretion were normal.

Conclusion: Our work for the first time enlightens a role for SERCA3 in a platelet function, the $\alpha_{\text{IIb}}\beta_3$ integrin-independent secretion of dense granules and ADP.

Disclosure of Interest: None Declared.

PO445-WED

CD32 antibodies protect FCGR2A mice from IGG mediated hypersensitivity reactions and thrombosis

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Background: CD32a activation by IgG immune complexes (IC) mediates thrombosis and shock in CD32A transgenic (Tg) mice. FCGR2A mice carry the CD32A transgene and are immune-competent, whereas endogenous IgG receptor (FcγR) knockout mice crossed with CD32A Tg mice are selectively immunodeficient. Hypersensitivity Reactions (HR) to IgG are mediated by FcγRs such as CD32a and are Type II (HR-T2; cell-bound IgG) or Type III (HR-T3; IgG IC). Certain IgGs induce in CD32A mice either HR-T2 reactions and thrombocytopenia without thrombosis, or HR-T3 reactions with concomitant thrombosis. CD32a monoclonal antibody (mAb) blockade, such as by IV.3, could prevent such reactions, but induces anaphylaxis in immunodeficient CD32A Tg mice, suggesting mAb CD32a targeting is not feasible.

Aims: (1) to determine whether immune-competent CD32A Tg mice react to CD32a mAbs as do immunodeficient CD32A Tg mice and whether such reactions mechanistically are HR-T2 or T3; (2) to test the effect of CD32a blockade by mAbs on IgG IC-induced thrombotic shock.

Methods: Various CD32a mAbs were injected into FCGR2A mice. Mice were challenged with IgGs that induce thrombocytopenia with or without thrombotic shock. Platelets were counted before and after CD32a mAb injection or exposure to active IgGs. Animals were observed for hypothermia and shock symptoms. Lung thrombosis was assessed histologically.

Results: CD32a mAbs induced thrombocytopenia independently of and concomitantly with HR-T2 (but not T3) reactions without thrombosis in FCGR2A mice. Such reactions were not observed with corresponding aglycosyl CD32a mAbs, which also completely prevented IgG IC-induced thrombosis.

Conclusion: IgG-induced thrombocytopenic, thrombotic, and hypersensitivity reactions can be differentiated in FCGR2A mice. CD32 mAbs cause HR-T2 without thrombosis which is avoided by deglycosylation. Whole aglycosyl CD32a mAbs can thus be safely infused into CD32A Tg mice and completely prevent HR-T2 and T3, and thrombosis.

Disclosure of Interest: None Declared.

PO446-WED

Converging roles of platelets and CD32A in IGG-mediated hypersensitivity reactions

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Background: Injection of certain IgGs into CD32A transgenic mice causes hypersensitivity reactions (HR), which are thought to be largely neutrophil-driven. The presence of CD32a on platelets in these mice fundamentally alters the nature of HR by introducing complexities involving thrombosis and spleen function.

Aims: We set out to identify differential responses to anti-platelet antibodies vs. immune complexes in CD32A mice and to identify the impact of platelet depletion and splenectomy on IgG-mediated HR.

Methods: HR and thrombosis were evaluated following injection of monoclonal (mAb) or polyclonal (pAb) antibodies directed against platelet or soluble antigens, including: (1) IV.3 (mAb that binds platelet CD32a), and rabbit anti-mouse-platelet pAbs; (2) thrombotic IgG immune complexes (IC) consisting of CD40L+anti-CD40L mAb or $\beta_2\text{GPI}$ +anti- $\beta_2\text{GPI}$ pAb. These were injected into mice having or lacking CD32a, in some cases following platelet depletion or splenectomy. Reactions to these IgGs were assessed by signs of HR and by measurement of core body temperature, platelet counts, and pulmonary thrombosis.

Results: IV.3 caused HR but not thrombosis in CD32A mice which was markedly suppressed by platelet depletion, suggesting platelets are primary mediators of IV.3-induced HR in this model. IV.3 caused HR similarly in control or splenectomized CD32A mice, suggesting splenic involvement is not required. Rabbit anti-mouse platelet pAbs caused HR in CD32A but not WT mice, suggesting a requirement for CD32A. Finally, platelet depletion significantly blunted both IC-induced HR and thrombosis.

Conclusion: These data indicate that platelets per se, and in particular platelet CD32a, play primary roles in IgG-mediated hypersensitivity reactions in CD32A transgenic mice. They also highlight the importance of including platelets in the analysis of hypersensitivity reactions in mouse models, underscoring the value of CD32a in translating mouse model results to human pathology.

Disclosure of Interest: None Declared.

PO447-WED

Plant food cyanidin-3-glucoside induces human activated platelet apoptosis via BCL-2/BCL-XL intrinsic pathway

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Background: Platelet apoptosis have been reported to be involved in patients with CVDs. Anthocyanins are major phytochemicals abundant in plant food and have been shown to play a protective role against CVDs. Our previous studies demonstrated that anthocyanins significantly inhibited platelet activation, aggregation and thrombus growth, however the effects and mechanisms of anthocyanin on platelet apoptosis haven't been explored.

Aims: To explore the effects of anthocyanin on platelet apoptosis via BCL-2/BCL-XL intrinsic apoptotic pathway.

Methods: Cyanidin-3-glucoside (Cy-3-g), the predominant bioactive compound of anthocyanins, was obtained from Polyphenol AS Company. Purified gel-filtered platelets from healthy volunteers were incubated with Cy-3-g (0.5, 5 and 50 μM) or PBS buffer as a control. The platelets were triggered by thrombin for inducing apoptosis. $\Delta\psi\text{m}$ and PS exposure were assessed by flow cytometry. Cytochrome C release,

activation of caspase-3, 8, 9, cleavage of gelsolin, the levels of anti-apoptotic proteins such as BCL-2, BCL-XL and proapoptotic proteins Bax, Bak, Bad, Bid and tBid in both activated and resting platelets were measured by western blotting.

Results: Cy-3-g at 5 and 50 μ M directly significantly induced $\Delta\Psi$ m dissipation, cytochrome C release, PS exposure and the cleavage of gelsolin in activated platelets dose dependently compared to control. The pro-caspase-8 and 9 were decreased, but activation of caspase-3, 8 and 9 was induced in activated platelets by 5 and 50 μ M Cy-3-g. However, Cy-3-g didn't induce the above changes in resting platelets. Cy-3-g significantly inhibited the expression of BCL-2, BCL-XL and increased the levels of Bax, Bak, Bad and Bid in activated platelets dose dependently, and no significant difference was observed in resting platelets.

Conclusion: Our data demonstrate for the first time that purified anthocyanin Cy-3-g directly accelerated apoptosis in activated platelets via the BCL-2/BCL-XL pathway.

Disclosure of Interest: None Declared.

PO448-WED

Modulation of actin cytoskeleton on shear-dependent fibrillogenesis of fibronectin

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Background: Fibril formation of fibronectin (Fn) is cell-mediated. Less is known about the contribution of biomechanical forces on unfolding of Fn.

Aims: This study aimed at investigating conformational changes of Fn, as induced by platelet (plt) integrins, cytoskeletal forces and/or shear rates simulating venous or arterial flow conditions.

Methods: Soluble Fn (100 μ g mL⁻¹), in the presence or absence of washed plts (2.5x10⁷/ml), was exposed to shear (stepwise increasing from 50 to 5000 s⁻¹). In parallel experiments, washed plts were pre-incubated with actin-modifying reagents, jasplakinolide (1 μ M) or cytochalasin D (10 μ M). For microscopic analysis, fluorescently labeled Fn was used. To study the structure of Fn fibrils, solutions were examined by laser scanning microscopy after shear exposure. To quantify the amount of fibril formation, deoxycholate solubility assays and densitometric analysis of Western blots were performed. Control experiments were conducted under static conditions.

Results: Microscopic analyses showed that exposing Fn to shear resulted in fibril formation. Fn fibril diameter varied from 0.5 to 5 μ m. Observed fibrils were linked with each other and varied in length (from 50 to 300 μ m). Western blotting and densitometric analyses revealed that fibril formation of Fn (calculated as the ratio of insoluble to soluble Fn) increased from 0.009 (samples without plts) to 0.176 (samples with plts) ($P < 0.05$, $n = 3$). Incubation of plts with jasplakinolide, which stabilizes actin, caused an increase in fibril formation by 41%, as compared to samples with untreated plts ($P > 0.05$, $n = 3$). In contrast, disruption of actin by cytochalasin D resulted in a decrease by 86% ($P < 0.05$, $n = 3$). Under static conditions, no fibril formation was detected.

Conclusion: Our results indicate that the fibril formation of Fn in solution is modulated by shear conditions and cytoskeletal forces. Our system provides useful information regarding shear-induced alterations upon unfolding of Fn and the contribution of plts.

Disclosure of Interest: None Declared.

PO449-WED

Janus kinase 3 (JAK3) dependent signaling is critical to Ca²⁺-dependent platelet activation and thrombus formation

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Background: Platelet adhesion to subendothelial collagen after plaque rupture results in increase of cytosolic Ca²⁺ activity followed by platelet activation, aggregation and degranulation. Janus kinase 3 (JAK3) is a member of the Janus family protein-tyrosine kinases which is strongly expressed in platelets, whereas lack of JAK3 exhibit a profound and early block in both B and T cell development. Nevertheless the impact of JAK3-dependent signaling in platelet activation remains unclear.

Aims: The present study explored whether Janus Kinase 3 participates in the regulation of platelet cytosolic Ca²⁺ activity, platelet activation and thrombus formation.

Methods: Fura-2-AM spectrofluorometry, ATP-release, aggregation, flow cytometry, fibrinogen binding, flow chamber, proteomics

Results: Compared to platelets from *jak3*^{+/+} mice, *jak3*^{-/-} platelets revealed a significant reduction in cytosolic Ca²⁺ increase as well as dense granule secretion upon stimulation with thrombin or CRP (collagen-related peptide). In addition, JAK3-deficient platelets showed an impaired agonist induced integrin $\alpha_{IIb}\beta_3$ activation and aggregation compared to wildtype platelets. Consequentially, the activation-dependent fibrinogen binding was significantly blunted in *jak3*^{-/-} platelets compared to *jak3*^{+/+} platelets. Moreover, the *in vitro* thrombus formation was significantly reduced with blood from *jak3*^{-/-} mice compared to blood from wildtype littermates. Finally, proteomic approaches were performed to investigate the underlying molecular mechanism and to identify potential downstream targets of Janus kinase 3 in platelets.

Conclusion: The present observations unraveled JAK3 as a novel powerful regulator of platelet integrin $\alpha_{IIb}\beta_3$ activation, platelet dense granule secretion and thrombus formation. JAK3 is at least partially effective in regulating the activation-dependent Ca²⁺ store release and extracellular Ca²⁺ influx in platelets.

Disclosure of Interest: None Declared.

PO450-WED

The identification and characterization of cinnamaldehyde analogs, as novel small molecule inhibitors of platelet-surface ERP57

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Background: The platelet-surface thiol isomerase ERp57 regulates platelet aggregation, granule secretion, integrin activation and thrombus formation *in vitro* and *in vivo*. Previous studies have examined the role of ERp57 using ERp57 activity blocking antibodies and to date, no small molecule inhibitors have been identified which specifically inhibit ERp57.

Aims: The aim of this study was to identify potential small molecule inhibitors of ERp57, verify their potency and selectivity, and test their effects on platelet function.

Methods: 3641 compounds were screened for ERp57 inhibitory activity using the insulin turbidity assay. 17 compounds were selected for efficacy and specificity follow-up studies, and related compounds were

purchased to perform SAR studies. Of these, a group of cinnamaldehyde analogs were revealed as potent inhibitors of ERp57 activity and the most potent compound, cinnamaldehyde-1 (CA1) was selected for further investigation in assays of platelet function including; platelet aggregation, granule secretion, calcium mobilisation and spreading on adhesive substrates.

Results: Incubation of washed platelets with CA1 (10–300 μ M) dose-dependently inhibited platelet aggregation, dense-granule and alpha-granule secretion in response to thrombin and U46619 stimulation. Calcium mobilisation from intracellular stores and platelet spreading on fibrinogen and CRP-XL were also diminished by all CA1 concentrations tested.

Conclusion: In this study we have identified a specific small molecule inhibitor of ERp57 and demonstrated its inhibitory effect on platelet-surface ERp57. Its effects on platelet function are similar to the observed effects of anti-ERp57 antibodies. The effects of CA1 on thrombus formation are currently being investigated to determine the potential for CA1 to reduce thrombosis. The identification of small molecule inhibitors such as CA1 provides additional means to explore the role of ERp57 in thrombus formation and may prove useful for the development of future antithrombotic therapies.

Disclosure of Interest: None Declared.

PO451-WED

Polyphosphate decay in serum is chemical, not enzymatic

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Background: Inorganic polyphosphates (polyP), widespread in biology, are stored with divalent metal ions in acidic subcellular organelles. PolyP is a major component of dense granules of human platelets and potentially modulates blood clotting and inflammation. Once released, polyP decays in human serum or plasma with a half-life of ~90 min, thereby limiting its action *in vivo*. This work was funded through the NIH.

Aims: Identify the enzymes in human serum responsible for polyP degradation.

Methods: We designed a high-throughput assay for polyP degradation by capturing biotinylated polyP on streptavidin-coated 96-well plates. Wells were incubated with serum or plasma, then washed with high salt to remove bound protein. Wells were then incubated with thrombin (a polyP-binding protein). After washing, bound thrombin was quantified, thereby revealing the level of polyP remaining in the well.

Results: PolyP decayed in serum or hirudin-anticoagulated plasma with an ~90 min half-life and this decay was resistant to a wide variety of phosphatase inhibitors, excluding EDTA. PolyP decay was restored in Chelex-treated serum by adding Ca²⁺ but not other metal ions. PolyP decayed at the same rate in boiled serum, indicating the decay was likely not caused by serum proteins. We further found that polyP decayed with an ~90 min half-life when incubated in pH 7.4 buffers containing plasma concentrations of free Ca²⁺. Calcium-mediated polyP degradation was highly pH dependent: higher pH increased the decay rate, while acidic conditions protected against Ca²⁺-mediated polyP hydrolysis.

Conclusion: PolyP decay in serum or plasma is dominated by Ca²⁺-mediated chemical hydrolysis, providing an intrinsic, chemical mechanism for limiting polyP function extracellularly. Our findings also provide an explanation for the acidic nature of organelles throughout biology in which polyP and metal ions are stored together (often referred to as 'acidocalcisomes').

Disclosure of Interest: None Declared.

PO452-WED

Effect of PAR-4 tethered-ligand peptide analogs on human platelet activation

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Background: Thrombin activates human platelets via protease-activated receptors (PARs) -1 and -4 by cleaving their extracellular *N*-terminal domain and generating a new tethered-ligand terminus that binds to the receptor. PAR-1 antagonists have been developed and used in clinical trials, however the development of specific PAR-4 antagonists suitable for clinical use is still under investigation.

Aims: We performed structure-function analysis of synthetic PAR-4 tethered-ligand peptide analogs in order to elucidate amino acid residues primarily responsible for interactions with the receptor.

Methods: The following peptides were synthesized: 1.AYPGKF, 2.GYPGKF, 3.GYPGQV, 4.Ac-AYPGKF, 5.*trans*-cinnamoyl-AYPGKF, 6.YPGKF, 7.Ac-YPGKF, 8.*trans*-cinnamoyl-YPGKF and 9.caffeoyl-YPGKF. Their effect on human platelet activation was studied by aggregometry (platelet-rich plasma; PRP and washed platelets) and flow cytometry (membrane expression of integrin $\alpha_{IIb}\beta_3$ and P-selectin). Computational studies for molecular and homology modeling were performed using appropriate software.

Results: Peptides 1 and 2 induce aggregation in PRP (EC₅₀ = 51.5 μ M and 682.5 μ M respectively) and in washed platelets (EC₅₀ = 26.2 μ M and 657 μ M respectively), expression of $\alpha_{IIb}\beta_3$ (EC₅₀ = 18.2 μ M and 17 μ M respectively) and P-selectin (EC₅₀ = 26.2 and 38.2 μ M respectively). Peptide 8 potently inhibits platelet activation induced by peptide 1. All the other peptides were inactive. Computational studies revealed that the methyl-group of alanine in peptide 1 interacts with residues Y183, A262, L333 and L357, which form a hydrophobic pocket within PAR-4, whereas the *trans*-cinnamoyl- conjugate of this peptide (peptide 8) interacts with residues Y183, Y187, A234, L237.

Conclusion: Alanine is critical for the agonistic activity of peptide 1 through PAR-4. Alanine substitution with *trans*-cinnamoyl-group, results in a peptide that interacts with residues that are not involved in PAR-4-mediated platelet activation and it further prevents agonist interaction with the receptor.

Disclosure of Interest: None Declared.

PO453-WED

Platelet HSP70 regulates integrin $\alpha_{IIb}\beta_3$ activation and platelet aggregation

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Background: The molecular chaperone heat shock protein 70 (Hsp70), in complex with Hsp90, STI1 and other proteins, plays diverse roles in cellular and organismal function. While Hsp70 is known to reside intracellularly in platelets as well as on the platelet surface and to be released from exosomes on platelet activation, specific roles for Hsp70 in platelet function remain unspecified.

Aims: We aimed to characterize a role for Hsp70 in platelet function and hemostasis. Our study was designed to test the hypothesis that the Hsp70 chaperone system regulates platelet function through control of platelet integrin $\alpha_{IIb}\beta_3$ activation.

Methods: *In vitro* biochemical, microscopy, flow cytometry, aggregometry and clot retraction assays of platelet function as well as *ex vivo* analyses of platelet aggregate formation in whole blood under shear were carried out in the presence of Hsp70 inhibitors, including VER-155008 and MKT-077.

Results: Hsp70 inhibition significantly decreased the spreading of purified human platelets on fibrinogen-coated but not collagen-coated cover glass, suggesting a role for Hsp70 activity in platelet integrin $\alpha_{IIb}\beta_3$ activation. Inhibition of platelet Hsp70 reduced platelet $\alpha_{IIb}\beta_3$ activation downstream of the collagen receptor GPVI, as Hsp70-inhibited platelets showed reduced PAC-1 expression and fibrinogen binding as well as P-selectin exposure and aggregation following stimulation with collagen-related peptide (CRP). In clot retraction assays, a measure of platelet $\alpha_{IIb}\beta_3$ activation and fibrinogen binding, Hsp70 inhibition delayed the retraction of washed platelets. *Ex vivo*, inhibition of Hsp70 in whole human blood prevented the formation of platelet aggregates on collagen under shear.

Conclusion: We find that Hsp70 inhibition diminishes platelet activation by preventing inside-out integrin $\alpha_{IIb}\beta_3$ activation. Overall, our results suggest an emerging role for Hsp70 in the regulation of inside-out platelet integrin $\alpha_{IIb}\beta_3$ activation, platelet-platelet aggregation, and ultimately thrombus formation.

Disclosure of Interest: None Declared.

PO454-WED

Influence of pro-MMP-9 on platelet aggregation and adhesion to fibrinogen

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Background: The experiments using molecular and pharmacological inhibitors have shown that the active form of MMP-9 inhibits platelet aggregation. Those experiments were performed on platelets washed from plasma which is a huge reservoir of pro-MMP-9. It is known that platelets greatly increase the synthesis and secretion of MMP-9 by monocytes, endothelial cells and some tumor cells which can focus the matrix-degrading activity of this enzyme to the particular sites of platelets adhesion and aggregation.

Aims: Study of the influence of pro-MMP-9 on platelets suspended in autologous plasma.

Methods: To provide platelets with conditions similar to those prevailing *in vivo*, platelet function was assessed in PRP. The influence of MMP-9 (final concentration 10–1000 ng mL⁻¹ simulating its local increase) on platelet function was examined using light transmission aggregometry and the microtiter immunoassay assessing the static adhesion to fibrinogen. In aggregation studies, 0.15 U mL⁻¹ thrombin, 2.5 mM ADP, 2 µg mL⁻¹ collagen, and 0.5 mM arachidonic acid were used as agonists. In adhesion studies, the influence of MMP-9 on adherence to fibrinogen of activated (thrombin with a final activity of 0.05 U mL⁻¹) or non-activated platelets was assessed immunologically.

Results: The pro-MMP-9 had no significant influence on the aggregation regardless of the agonist used but had a dose-dependent inhibitory influence on platelet adhesion to fibrinogen. The inhibition was stronger when activated platelets were used (39.9%–57% decrease of the adhesion of the control PRP vs. 9.2%–41% decrease for non-activated platelets, n = 12). Interestingly, o-phenantroline which is a selective inhibitor of metalloproteinases on platelet adhesion, did not reverse the inhibitive influence of MMP-9 on platelet adhesion.

Conclusion: The inhibitory effect of MMP-9 on platelet adhesion does not depend on its enzymatic activity and probably involves binding of MMP-9 to the receptor for fibrinogen.

Disclosure of Interest: None Declared.

PO455-WED

The defective CD36 gene mutations cause a molecular expression diversity on platelets and monocytes even in non-deficient phenotype population

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Background: CD36 is a multifunctional glycoprotein expressed on various human cells, including platelets and monocytes. Five CD36 gene mutations (C268T, 949insA, 329-339del, 1228-1239del and 629-631del/insAAAAC) are mainly responsible for CD36-deficient phenotypes in Japan. It has also reported that platelet CD36 expression varies widely among normal phenotype individuals.

Aims: In order to obtain further insight into CD36 expression, we investigated the association between platelet and monocyte CD36 expression levels and defective mutations in the Japanese population.

Methods: Blood samples were collected from 135 healthy Japanese volunteers. CD36 expression levels on platelets and monocytes were quantitatively analyzed by flow cytometry. Real-time PCR, PCR-RFLP and allele-specific PCR were performed to detect mutant genotypes.

Results: In this population, we found 2 (1.5%) and 9 (6.7%) CD36-deficient subjects as type I and type II, respectively. Among normal phenotype subjects, CD36 expression levels ranged from 1,259 to 11,002 (4,487 ± 2,017) molecules/platelet and from 211 to 5,150 (1,628 ± 986) molecules/monocyte. Genotyping assay showed that heterozygotes with the defective mutations were present in normal (12.9%) and type II-deficient (66.7%) subjects, and that these heterozygous mutations led to decreases in CD36 surface expression on platelets and monocytes.

Conclusion: Heterozygous CD36 mutations, previously known to lead to deficiency in this molecule, are one of the factors responsible for the diversity of CD36 surface expression levels on platelets and monocytes in normal phenotype subjects.

Disclosure of Interest: None Declared.

PO456-WED

Shear-induced fibrillar-like supramolecule of plasma fibronectin: a new form of fibronectin with enhanced activity in platelet adhesion and aggregation

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Background: We reported on the formation of fibronectin fibrils (FN fibrils) induced by shear rates.

Aims: Here, we characterized the function of shear-induced FN fibrils in platelet adhesion and aggregation.

Methods: For adhesion experiments, CMFDA labeled platelets were placed onto immobilized FN or FN fibrils (25 µg mL⁻¹) for 30 min at 37°C. In parallel experiments, platelets resuspended in FN-depleted plasma were placed onto immobilized FN, collagen, or fibrinogen

(10 $\mu\text{g mL}^{-1}$) in the presence of soluble FN (300 $\mu\text{g mL}^{-1}$) or FN fibrils (10 $\mu\text{g mL}^{-1}$). For aggregation experiments, FN (5, 10, 300 $\mu\text{g mL}^{-1}$) or FN fibrils (5, 10 $\mu\text{g mL}^{-1}$) was added to PRP or platelets resuspended in FN-depleted plasma. Aggregation was induced by 400 nM PMA or 10 $\mu\text{g mL}^{-1}$ collagen.

Results: The adhesion rates of washed platelets were higher onto surfaces coated with FN fibrils than with plasma FN. In parallel experiments using platelets resuspended in FN-depleted plasma, addition of plasma FN (300 $\mu\text{g mL}^{-1}$) increased platelet adhesion onto immobilized FN (from 0.14 ± 0.01 to 0.18 ± 0.02 , $P = 0.04$), collagen (from 0.14 ± 0.005 to 0.2 ± 0.01 , $P = 0.0007$), and fibrinogen (from 0.16 ± 0.03 to 0.22 ± 0.01). Addition of FN fibrils (10 $\mu\text{g mL}^{-1}$) had a similar supportive effect. Conversely, FN showed an inhibitory effect in platelet aggregation. Activation by PMA induced aggregation of PRP by 81%. In the presence of plasma FN at 5, 10, 300 $\mu\text{g mL}^{-1}$, platelet aggregation was reduced to 50%, 41%, and 29.5%, respectively. A stronger inhibition on platelet aggregation was seen with FN fibrils. PRP aggregated by 35.4% and 17% in the presence of 5 and 10 $\mu\text{g mL}^{-1}$ FN fibrils, respectively. The same phenomenon was observed in aggregation assays using platelets resuspended in FN-depleted plasma and collagen as activating agonist.

Conclusion: Our study suggested that shear-induced FN fibrils have stronger activity in supporting platelet adhesion and inhibiting platelet aggregation than normal plasma FN. This finding emphasizes the importance of FN assembly on its activity in platelet function.

Disclosure of Interest: None Declared.

PO457-WED

Elastin-derived peptides regulate leukocyte-platelet aggregates

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Background: Elastin is a major structural extracellular matrix component of the arterial wall providing the elastic recoil properties and resilience essential for proper vascular function. Vascular remodeling is associated with elastin fragmentation and generation of elastin-derived peptides (EDP). We recently reported that EDP regulate thrombosis by decreasing platelet aggregation and delaying time for arteriole occlusion in a thrombosis mouse model (Kawecki et al, *ATVB*. 2014). Leukocytes, mainly monocytes and polymorphonuclear neutrophils (PMN), are also key actors involved in thrombosis and prothrombotic/procoagulant leukocyte-platelet aggregate (LPA) formation.

Aims: In this study, we evaluated the effects of EDP, derived from organo-alkaline hydrolysate of bovine insoluble elastin (kappa-elastin, kE), on 1) LPA formation, using flow cytometry, and 2) monocyte expression of tissue factor (TF), using both western blot (WB) and thrombin generation assay (TGA).

Methods: 1) LPA were induced by 10 μM TRAP or a mixture of 20 μM ADP/10 $\mu\text{g mL}^{-1}$ collagen in human whole blood, and identified as CD45-PE/CD41-FITC double positive population. 2) Human monocytes were purified by elutriation and stimulated by LPS 100 or 10 ng mL^{-1} for TF expression assessment by WB and TGA, respectively.

Results: We did not observe any significant effect of kE (100 $\mu\text{g mL}^{-1}$) on agonist-induced percentage of LPA. On the contrary, the MFI of CD41-FITC was significantly decreased by about 20% for both monocyte- and PMN-platelet aggregates induced by TRAP ($n = 10$) and ADP/collagen ($n = 5$). Concerning the effect of kE on TF monocyte expression, preliminary results showed that kE increased LPS-induced monocyte TF expression as shown by WB and TGA.

Conclusion: These results suggest that EDP regulate LPA formation in whole blood by decreasing the size of LPA. Experiments are currently ongoing to determine the functional consequences on the prothrombotic and procoagulant activities of these LPA.

Disclosure of Interest: None Declared.

PO458-WED

Platelet dense granule secretion defects may obscure our understanding of A-granule secretion mechanisms: evidence from MUNC13-4-deficient platelets

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Background: Platelets release a wide array of bioactive molecules from their α -granules, which may have roles in thrombosis, haemostasis, inflammation, wound healing, angiogenesis and malignancy. Platelets from genetically modified mice have been instrumental in revealing the molecular mechanisms of α -granule secretion. The SNARE regulator, Munc13-4, is an essential factor for dense granule secretion. *Unc13d*^{tmx} platelets (which lack Munc13-4) have a substantial, but incomplete, suppression of α -granule secretion.

Aims: To understand the regulation of α -granule secretion.

Methods: Washed platelets from wildtype (WT) or *Unc13d*^{tmx} mice were stimulated with PAR4-activating peptide (PAR4-AP), and surface expression of CD62P was used as a marker of α -granule secretion.

Results: PAR4-AP-induced CD62P expression was significantly less in *Unc13d*^{tmx} platelets compared to WT. However, this block to α -granule secretion was substantially 'rescued' by the addition of ADP. Therefore, there must also be a Munc13-4-independent pathway that is capable of inducing substantial α -granule secretion. In the presence of the P2Y₁₂ antagonist AR-C69931MX there was a marked suppression of CD62P surface expression, in both WT and *Unc13d*^{tmx} platelets, and the ADP-mediated rescue of secretion was ablated. The P2Y₁ antagonist, MRS2279, had no effect. Therefore, secreted ADP enhances Munc13-4-independent α -granule secretion through the P2Y₁₂ receptor, but the presence of this pathway is obscured by the complete loss of dense granule secretion in *Unc13d*^{tmx} platelets.

Conclusion: ADP and subsequent P2Y₁₂ signalling enhance α -granule secretion. Moreover, disruption of dense granule secretion can alter the apparent contribution of different proteins to α -granule secretion. We believe that our data raise the more general question: How much do we really know about the molecular mechanisms of α -granule release in platelets?

Disclosure of Interest: None Declared.

PO459-WED

The inositol(1,4,5)trisphosphate receptor type 2 is important in thrombin induced platelet activation and is required for thrombus formation *in vivo*

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Background: Ca^{2+} elevation is essential in platelet activation. The inositol trisphosphate receptors (IP₃R) are intracellular Ca^{2+} release channels. There are 3 types of IP₃Rs. Platelets express IP₃R1 and IP₃R2 abundantly (IP₃R1 > IP₃R2) with the type 3 being least expressed. The role of each receptor in Ca^{2+} signalling has not been elucidated.

Aims: The aim of this study was to explore the role of the IP₃R2 in platelet function using platelets from IP₃R2 knock out (KO) mice. IP₃R2 KO mice are fertile and show no apparent phenotype.

Methods: Platelets from IP₃R2 KO and wild type (WT) litter mates were isolated and examined for aggregation responses and Ca²⁺ elevation. Bleeding time studies and effects of ferric chloride application in mesenteric arterioles were carried out

Results: Platelets from WT mice showed good expression of type I and type II IP₃R and other platelet proteins. Platelets from IP₃R2 KO mice show no expression of IP₃R2 protein, normal expression of IP₃R1 and other proteins. In vitro aggregation responses to all agonists tested have been found to be normal except for responses to low dose thrombin. Ca²⁺ signalling responses using Fura2-PE labelled platelets also showed no difference in responses to agonists tested except for low dose thrombin where the responses were reduced. In bleeding time analysis we found no difference between WT and KO mice suggesting no effect on haemostasis. We further examined ferric chloride application in mesenteric arterioles where thrombin generation may be important. In WT mice exposure of the mesenteric arterioles to ferric chloride led to reduced blood flow and occlusion of the vessel. In IP₃R2 KO mice there was no occlusion of the vessels suggesting a protection against thrombus formation.

Conclusion: The IP₃R2 Ca²⁺ release channel is important in thrombin stimulated platelet activation and thrombus formation. Our studies suggest it to be a suitable target to prevent vessel occlusion but without the side effects of bleeding.

Disclosure of Interest: None Declared.

PO460-WED

Transcription of exogenous RNA in human platelets

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Background: Platelets are involved in many physiological and pathological processes, including hemostasis, thrombosis, and inflammation. Despite this wide range of functions, platelets do not have nuclei and are therefore unable to regulate gene expression at the transcriptional level. Modifying gene expression in platelets requires manipulation of nucleated precursor cells, which is tedious and impractical for transfusion purposes.

Aims: In a step towards altering gene expression in transfusable platelets, we hypothesize that platelets can be directly genetically modified with liposomes capable of RNA transcription. The goal of this study was to test whether RNA-transcribing liposomes can be delivered to platelets, leading to upregulation of an exogenous, reporter mRNA.

Methods: Transcriptional components, including a reporter DNA template, T7 RNA polymerase, and ribonucleotides, were encapsulated within liposomes and incubated with human platelets *ex vivo*. Internalization of RNA-transcription liposomes was measured using various techniques, including flow cytometry, fluorescence microscopy, and quantitative PCR.

Results: We have shown that reporter mRNAs are transcribed within ~200 nm purified liposomes. These liposomes are internalized by human platelets *ex vivo*, leading to accumulation of functional reporter mRNA within platelets.

Conclusion: This initial proof-of-concept study represents a method to directly alter RNA levels in anucleate platelets, which can have important applications in studying platelet biology or for therapeutic use. Future studies will explore the use of therapeutically relevant gene targets, as well as external stimuli to initiate transcription, allowing for controlled modification of gene expression in human platelets.

Disclosure of Interest: None Declared.

PO461-WED

The PI(3,4,5)P₃-binding protein Rasa3 negatively regulates Rap1B and integrin α IIb β 3-mediated spreading

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Background: Phosphoinositide 3-kinase (PI3-kinase) is a lipid kinase that phosphorylates the 3-position on PI(4,5)P₂ generating PI(3,4,5)P₃ and plays an important role in integrin regulation and platelet function. The mechanism by which PI3-kinase modulates platelet function, however, is still largely unknown. We recently used an unbiased proteomic approach to identify Rasa3 (GAP1^{IP4BP}) as a major PI(3,4,5)P₃-binding protein in human platelets. Rasa3 is a GTPase-activating protein (GAP) that has dual activity towards the small G-proteins Ras and Rap.

Aims: The aim of this study was to characterise the role of Rasa3 in integrin α IIb β 3 regulation and platelet function.

Methods: Rap1-GTP or Ras-GTP levels were determined by RalGDS or Raf1 pull-down experiments, respectively. Localization studies were performed on platelets using immunofluorescence and confocal microscopy. Rasa3 constructs were transfected into CHO cells expressing integrin α IIb β 3 and PAR1. CHO cell spreading on fibrinogen was quantified by phalloidin staining.

Results: Platelet activation with thrombin resulted in Rasa3 phosphorylation and rapid activation of both Ras and Rap in human platelets. Rap1 activation, but not Ras activation, was largely dependent on PI3-kinase. Rasa3 strongly co-localized with integrin α IIb β 3 in human platelets, with thrombin inducing a significant increase in membrane localization of Rasa3, integrin α IIb β 3 and Rap1. Rasa3 expression in CHO cells caused a reduction in both basal and PAR1-mediated Rap1 activation, however mutation of Rasa3 to RapGAP deficient forms restored Rap1-GTP levels. This effect correlated with the finding that integrin α IIb β 3-mediated spreading was lost in CHO cells expressing wild-type Rasa3, but not RapGAP deficient Rasa3.

Conclusion: Our results show that Rasa3 phosphorylation and membrane localization are altered by platelet stimulation, and that Rasa3 negatively regulates integrin α IIb β 3-mediated spreading through the inhibition of Rap1.

Disclosure of Interest: None Declared.

PO462-WED

Dextran sulfate triggers platelet aggregation via PEAR1, independent from Syk

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Background: Dextran sulfate (500K; DxS₅₀₀) was originally evaluated as an anticoagulant with heparin-like properties. Recently, DxS₅₀₀ was found to induce fibrinogen receptor (α IIb β 3) activation through a Syk-independent Src/PI3K/Akt-dependent tyrosine kinase pathway in both human and murine platelets. The platelet surface receptor(s) however, responsive to DxS₅₀₀, has not been identified. PEAR1-signalling sustains the activation of α IIb β 3 in aggregating platelets via Src/PI3K/Akt, favoring the formation of stable platelet aggregates.

Aims: Since both DxS₅₀₀ and PEAR1 induce sustained platelet aggregation via the same underlying signalling pathways in platelets, we investigated whether DxS₅₀₀ platelet aggregation is mediated via PEAR1.

Methods: cfr results.

Results: Dxs₅₀₀ induced a dose-dependent phosphorylation of PEAR1 in static (unstirred) human platelets (P-Tyr; Western blot). This Dxs₅₀₀-induced PEAR1-phosphorylation was largely eliminated in the presence of excess of soluble pentameric PEAR1 and after blocking PEAR1 by its monomeric ligand FcεR1α. Dxs₄ (4K; short strains) did not aggregate human platelets nor induced PEAR1-Phosphorylation. This is compatible with the need of PEAR1-multimerisation in triggering PEAR1-activation. Murine platelet Pear1 was also phosphorylated by Dxs₅₀₀, resulting in downstream phosphorylation of Akt. Static incubation of WT vs. *Pear1*^{-/-} platelets with Dxs₅₀₀ showed a 8-fold decrease of Akt-P in the KO-platelets, whereas Syk-P was not affected. The Dxs₅₀₀-induced aggregation was reduced by 50% in *Pear1*^{-/-} vs. WT platelets, suggesting that the Pear1/Akt- and Syk-pathways are complementary in Dxs₅₀₀-aggregation, as confirmed by Syk-inhibition.

Conclusion: We demonstrate that Dxs-induced platelet aggregation – both in human and murine platelets – is mediated via activation of Akt through PEAR1, independent of Syk.

Disclosure of Interest: None Declared.

PO463-WED

Recombinant FVIII and FVIIa co-localize with platelet aggregates adherent to a collagen surface exposed for flowing normal or hemophiliac blood, and promote local fibrin deposition

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Background: Mural platelet thrombi are considered to facilitate hemostasis in hemophilia via rFVIII/ rFVIIa binding to platelet membranes with enhancement of local fibrin deposition.

Aims: To show that Factors VIII and VIIa co-localize to platelet membranes of adherent platelet aggregates and promote local deposition of fibrin.

Methods: We collected blood from 9 patients with severe hemophilia A just prior to a prophylactic dose of rFVIII, and from 8 normals, into a plastic tube containing 5 U/ml FC low MW heparin, 1.75 µg/ml of the Tab (anti-CD41) mAb antibody, and 1.0 µg/ml of ALEXA 555-conjugated rabbit anti-mouse secondary antibody, plus 0, 11, 33 or 100 ng/ml rFVIII-Oregon green. Similar approaches were used for rFVIIa or fibrin. Blood at 37° C was withdrawn at 270 sec-1 through a flow chamber, over 150 µm glass cover slips with preadsorbed microfibrillar collagen, using epifluorescence video microscopy and digital image analysis (MetaMorph Premier software).

Results: Added rFVIII or rFVIIa co-localized with platelet membranes and increased with rFVIII concentration. With hemophiliac blood and 100 ng mL⁻¹ rFVIII, percent co-localization at 7 min was 52.6 ± 7.7 (N = 4). Platelet aggregate growth rate, image pixels/min, was 26% lower in hemophiliac blood, but increased (*P* = 0.0001) 2.34 ± 0.62 (6)-fold with rFVIII, and (*P* = 0.0005) 1.91 ± 0.56 (6)-fold with 1500 ng mL⁻¹ rFVIIa. Fibrin deposition was delayed by 3–5 min in normal blood, but by >11 min in hemophiliac blood. Addition of rFVIII or rFVIIa shortened the latter time to 7–9 min. Fibrin appeared as comet-shaped deposits (comet head 20–25 µm in diameter) centered over each platelet aggregate (3–10 µm), the comet tail trailing 60–75 µm downstream.

Conclusion: In flowing whole blood in real time, these data show that rFVIII and rFVIIa co-localize with platelet membranes of activated, surface-adherent platelet aggregates, promoting local fibrin deposition.

Disclosure of Interest: None Declared.

PO464-WED

Identification of low molecular weight factors released from platelets that induce procr in monocytes

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Background: Endothelial protein C receptor (EPCR) regulates haemostasis by supporting activation of Protein C. EPCR is primarily expressed on endothelial cells but previous studies have shown EPCR mRNA (*procr*) can also be induced in monocytes by GPVI-activated platelets.

Aims: To investigate the mechanism by which platelets regulate monocyte *procr* expression.

Methods: Platelets activated by the collagen peptide mimetic CRP-XL were allowed to interact with monocytes, then isolated using immunomagnetic beads, and relative expression of *procr* was measured in monocytes by RT-qPCR. Platelet releasate was prepared from washed platelets and separated on the basis of MW. Proteins were subjected to tryptic digestion and peptides analysed by electrospray ionization mass spectrometry (ESI-MS) using a Q-Exactive Orbitrap mass spectrometer. Raw data was processed using Proteome Discoverer 1.4 to identify and quantify the releasate proteins.

Results: GPVI-activated platelets induced monocyte *procr* expression in a time-dependent manner, reaching maximum at 8 h (*P* = 0.0028; *n* = 4). Preventing direct platelet-monocyte interaction with a CD62P-blocking antibody 9E1 did not change *procr* expression indicating the active component was released from the platelets. This was confirmed by incubating isolated monocytes with platelet releasate. Analysis of fractionated platelet releasate identified proteins ~10 kDa as responsible for *procr* induction (*P* = 0.0192; *n* = 9). MS analysis identified several candidates ~10 kDa, including Platelet Factor 4 (PF4; MW 11kD), a molecule known to affect monocytes. Incubation of platelet releasate with heparin-agarose beads to remove PF4 removed the ability to induce *procr* expression in autologous monocytes compared to untreated releasate fraction (*P* = 0.0340; *n* = 3).

Conclusion: These results suggest that PF4 released from activated platelets induce expression of *procr* in monocytes. This may be a modifiable mechanism by which monocytes regulate coagulation within a clot.

Disclosure of Interest: None Declared.

PO465-WED

Cell surface expression of chemerin receptors on human platelets upon cell activation

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Background: Chemerin is a chemoattractant for human plasmacytoid dendritic cells and NK cells. Chemerin binds to Chemokine-like Receptor 1 (CMKLR1), G-protein Receptor-1 (GPR-1), and Chemokine Receptor-Like 2 (CCRL2) but only CMKLR1 and GPR-1, both members of the G-protein coupled receptor family, transduce cellular signals upon activation. We have previously shown that platelet releasate contains chemerin(s) with chemoattractant activity.

Aims: To characterize the expression patterns of the chemerin signaling receptors, CMKLR1 and GPR-1 in human platelets.

Methods: Human resting platelets were isolated from whole blood of healthy donors. Thrombin receptor activator peptide 6 was used to activate washed resting platelets. Levels of platelet activation were checked by expression of P-selectin (CD62p). Resting and activated platelets were analyzed either fresh or fixed, with or without permeabi-

lization by flow cytometry, using fluorescence conjugated CD41 (gpIIb), CD62p, CMKLR1 and GPR1 antibodies.

Results: Whether fresh or fixed, non-permeabilized resting platelets showed no detectable expression of the chemerin receptors, CMKLR1 and GPR-1, on their surface. However, GPR-1 expression was detected on the surface of non-permeabilized activated platelets whether fresh or fixed, in a pattern similar to CD62p. GPR-1 expression was always detected in resting or activated platelets when the platelets were fixed and permeabilized showing that it is in an intracellular compartment before activation. CMKLR1 expression can only be detected in either activated platelets or ones that had been fixed and permeabilized. An immunofluorescence study of human bone marrow smear revealed that megakaryocytes also expressed CMKLR1.

Conclusion: The chemerin signaling receptors, GPR-1 and CMKLR1, are expressed in the intracellular compartment of human platelets. Platelet activation leads to translocation of GPR-1 and CMKLR1 to their cell surface, where they may play a role in clot formation and/or platelet-related immune functions.

Disclosure of Interest: None Declared.

PO466-WED

GPIIb α activation mediates platelet tissue factor (TF) phosphorylation, transfer to lipid rafts (LR) and activity. TFPI and protein S, released by other agonists, would assemble the inhibitory complex (TF/FVIIa/TFPI/PS) in LR, dampening FXa activity

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Background: Platelet (P) GPIIb α plays a major role in clotting. Its activation includes transfer to lipid rafts (LR), and signaling through the SFKs. We observed that GPIIb α activation by VWF induces Tissue Factor (TF) activity, whereas activation with TRAP and collagen did not. TF activation is associated with cytoplasmic Cys253-Cys258 phosphorylation. Platelets also contain α -TFPI, which in HUVEC is membrane associated through glycosylphosphatidylinositol, suggesting association with LR. Platelets also secrete protein S (PS), a TFPI co-factor for TF/FVIIa/FXa inactivation.

Aims: To better understand the mechanisms of P-TF activation-inactivation.

Methods: P-TF activity: FXa generation assay (nM 2×10^{-7} washed platelets). WB, IP and Co-IP with mAbs recognizing phosphorylated TF serines (W. Ruf) and TFPI (G. Broze). PP2 and SU-6656 to assess the SFKs. LR-enriched fractions were obtained in sucrose gradients or in the pellets of 1% Triton X-100, 4°C, lysed centrifuged Ps.

Results: TRAP and Collagen, not VWF-Ristocetin (VWF-R) induced α - δ granules secretion of washed Ps. However, only VWF-R triggered >10-fold increase of FXa generation (medians of 17 and 182 nM 2×10^{-7} platelets, $P < 0.0001$), which is associated with TF Ser253-Ser258 phosphorylation and transfer to LR domains. FXa generation was dampened by α -GPIIb α , α -TF, α -FVIIa mAbs and by added TFPI and inhibitors of SFKs, mainly Lyn, and PI3K, but not of PKC or Syk. Lack of TRAP/collagen-induced FXa generation was explained by P-released TFPI and PS, because with these agonists FXa is generated by α -TFPI+ α -PS. Both VWF-R and TRAP, but not collagen, induced transfer of GPIIb α and phosphorylated TF to LRs. In contrast, TRAP and Collagen, but not VWF, induced expression in LRs of TFPI and PS, which co-pp with GPIIb α and TF.

Conclusion: P-TF activity is triggered by VWF-induced GPIIb α activation and would be rapidly dampened by concerted action of released TFPI and PS. The inhibitory complexes (TFPI/PS/TF/GPIIb α) for FVIIa and FXa would be assembled on LR domains.

Disclosure of Interest: None Declared.

PO467-WED

The impact of circulating histones on platelet activation and thrombus formation *in vivo*

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Background: Circulating histone levels are elevated in the setting of trauma and correlate with the severity of injury. Studies have suggested that histone toxicity may contribute to tissue damage, as well as the development of a coagulopathic state in a subset of trauma patients. In mouse models, histone infusion leads to thrombocytopenia, elevated cytokine levels, tissue damage and death.

Aims: In the present study, we sought to determine the impact of circulating histones on platelet function and thrombus formation *in vivo*.

Methods: Multiple sublethal doses of histones (10–45 mg kg⁻¹) were administered to mice via intravenous infusion. Intravital imaging was used to quantify platelet accumulation, activation and fibrin deposition following laser-induced vascular injury in cremaster arterioles.

Results: None of the histone doses tested had a significant effect on total platelet accumulation following laser-induced injury. The total area of P-selectin positive platelets and fibrin deposition were also not impacted by histone infusion. Interestingly, mice treated with the highest dose of histones (45 mg kg⁻¹) had a subset of platelets that were P-selectin positive upon initial adhesion to developing thrombi, suggesting these platelets were circulating in a pre-activated state. Further, thrombi in histone treated mice were significantly more stable over time than thrombi from vehicle treated mice.

Conclusion: Infusion of high doses of histones results in activation of a subpopulation of circulating platelets and increased thrombus stability. The mechanism(s) by which histones exert these effects remain to be determined, but these results suggest how abnormal platelet function may contribute to the altered hemostatic/thrombotic profile observed in some trauma patients.

Disclosure of Interest: None Declared.

Platelet disorders III

PO468-WED

Evaluation of ISTH-bleeding assessment tool in individuals with suspected inherited platelet function disorder referred to tertiary health care facility in Pakistan

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Background: ISTH bleeding assessment tool (ISTH-BAT) has been used in assessing bleeding symptoms in individuals with von Willebrand disease. However its utility in assessment of inherited platelet dysfunction is limited

Aims: To evaluate clinical utility of ISTH-BAT in predicting platelet defect in individuals with suspected inherited platelet dysfunction.

Methods: Individuals with bleeding symptoms and suspected inherited platelet dysfunction referred (from January 2013–June 2014) to Aga Khan University hospital, for platelet function testing were included. Healthy volunteers were taken as control. ISTH-BAT questionnaire was applied by hematology residents prior to lumiaggregometry

Results: Total of 191 individuals was studied (161 with suspected inherited platelet dysfunction and 30 healthy volunteers). Out of 161, platelet defect was found in 47 (29%) individuals of which Glanzmann thrombasthenia, Bernard Soulier syndrome and Quebec disorder constitute 33 (20.5%), 10 (6%) and 4 (2.5%) respectively. Healthy volunteers showed low ISTH-BAT score (median 0; interquartile range [IQR] 0–0). The ISTH-BAT score in individuals with suspected inherited platelet dysfunction with a platelet defect detected by lumiaggregometry

(median 4; IQR 8–3) was significantly higher than those with normal platelet function (median 2; IQR 3–1) ($P < 0.001$). The ISTH-BAT score was associated with a demonstrable platelet defect on platelet function testing (area under the receiver operating characteristic curve = 0.8001 [95% confidence interval (CI) 0.725–0.875, $P < 0.001$] and odds ratio 3.26 [95% CI 2.14–4.38, $P < 0.001$]. The sensitivity, specificity, positive predictive value and negative predictive value of ISTH-BAT in predicting the presence of a platelet defect on lumiaggregometry was 80.8%, 67.5%, 49% and 89.5% respectively.

Conclusion: ISTH-BAT is found to be a powerful tool for predicting the presence of a platelet defect on lumiaggregometry in patients with suspected inherited platelet dysfunction.

Disclosure of Interest: None Declared.

PO469-WED

Effect of cardiopulmonary bypass pump on the platelet function using platelet function analyzer (PFA-100) pre and postoperative cardiac surgery operation

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Background: Cardiac surgery operations are one of the leading causes to hemostasis alteration. The cause of postoperative bleeding that is related to platelet dysfunction is still unknown. Recurrent cardiovascular events after coronary artery bypass surgery due to platelet dysfunction.

Aims: Effect of Cardiopulmonary bypass pump on the platelet function pre and post op coronary artery bypass surgery using platelet function analyzers.

Methods: Blood samples at three time points will be drawn from 50 patients going for cardiopulmonary bypass (CPB). Before starting the CPB for every patient, we will collect 20 cc blood from peripheral access, one tube (EDTA tube) for complete blood count (CBC) and one tube (Sodium Citrate tube) for routine coagulation profile and one tube (Lithium Heparin tube) for platelet function tests. The second blood samples will be collected 60 min from the start of CPB with same samples for CBC, coagulation and platelet function. PFA-100 system consists of a cartridge in which a membrane and an aperture are coated with either collagen/adenosine-5 diphosphate or collagen/epinephrine. The instrument determines the time required for full occlusion of the aperture (closure time).

Results: A Platelet Function Analyzer 100 closure time less than 190 s was defined as residual platelet reactivity. In patients without complications, Primary homeostasis capacity in both collagen/adenosine-5-di-phosphate closure time (CADP-CT: 83 s pre-op, 78 s post-op and 74 s in the 1st day) and collagen/epinephrine closure time (CEPI-CT: 98 pre-op, 95 post-op and 85 s in the 1st day). In patients with post-op myocardial ischemia, increased PHC was determined by decreased CADP-CT (66 vs 83 s) than the uncomplicated patients.

Conclusion: Platelet reactivity is independently correlated with the worse clinical outcomes. However the PFA-100 should discover such conditions to help improving the clinical outcomes of those patients.

Disclosure of Interest: None Declared.

PO470-WED

Decreased platelet reactivity in women with heavy menstrual bleeding: a novel approach in the gynaecology clinic to diagnose thrombopathy using a novel platelet function test

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Background: Although heavy menstrual bleeding (HMB) is a common gynaecological problem, the pathophysiology has not been elucidated in the majority of these women. We studied the prevalence of thrombopathy in these women including the introduction of a novel platelet function test.

Aims: To study the contribution of thrombopathy to HMB using a whole blood platelet function test.

Methods: Fifty-eight women with HMB (median: 48.4 years, range 40–60 years) were enrolled into our study upon informed consent and after permission of the METC. None of these women took NSAIDs (< 24 h) before blood sampling. Detailed platelet function analysis was performed in whole blood within 30 min after blood sampling using flow cytometry analysis (FACSCalibur, BD). Platelet reactivity was assessed by whole blood analysis of platelet surface activated GPIIb-IIIa and P-selectin expression upon activation with several agonists, while vWF reactivity was assessed by vWF binding to platelets, using flow cytometry. Normal ranges for platelet reactivity by several agonists (adenosine diphosphate (ADP), collagen-related peptide (CRP), thrombin-receptor-associated peptide (TRAP) and ristocetin) were defined in 22 healthy persons (median: 34.1 years, range 24–55 years). Thrombopathy was defined as a decreased response upon activation below the mean -2* standard deviation (SD) of the controls. The 2*SD lower level control values were: 41% for ADP response, 60% for CRP response and 64% for TRAP and 48% for ristocetin response. Statistical analysis was performed by SPSS software package.

Results: We diagnosed thrombopathy in 29% (17/58) of the women with HMB with decreased platelet reactivity below 2*SD of the normal values upon activation with at least one of the agonists.

Conclusion: Our data show that thrombopathy is present in 29% of these women. This is remarkable high compared to the control group. Our platelet function test provide new insights in the pathophysiology of HMB and gives the gynaecologist better insight in the treatment of HMB patients.

Disclosure of Interest: None Declared.

PO471-WED

Diagnostic accuracy of flow cytometry analysis of platelet leukocyte aggregates in acute coronary syndrome patients vs. normal volunteers

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Background: Current challenges to use of platelet leukocyte aggregates (PLA) in diagnosis of acute coronary syndrome (ACS) include standardizing an assay, reporting data in a clinically relevant manner, establishing objectively defined cutoff values, and making flow cytometry available to the attending physician. Previous work has failed to address use of Receiver Operator Characteristic (ROC) curve analysis

to establish objective cutoffs, and the possibility that data collected by automated blood counters for a complete blood count (CBC) correlate with platelet leukocyte aggregate elevation in acute coronary patients.

Aims: This project aimed to collect data for a normal range of platelet leukocyte aggregates in normal non-ACS volunteers and acute coronary patients, present ROC curve analysis to compare to cardiac troponin I, and examine automated complete blood count data for PLA correlation.

Methods: All participants signed informed consent in accordance with local Helsinki ethics committee. In a single center between June 2014 and September 2014, platelet leukocyte aggregates were measured blindly in $n = 20$ consecutive acute coronary syndrome patients and $n = 35$ normal non-ACS volunteers by flow cytometry. CBC data on the same patients was collected from an Advia 2120i automated blood counter.

Results: Patients with acute coronary syndrome did have higher mean platelet monocyte aggregates and median fluorescent intensity of platelet markers on the monocyte cell population compared to normal non-ACS volunteers as hypothesized ($P < 0.05$). However, ROC curve analysis showed a lack of diagnostic utility, and no correlations with automated blood counts were found in the variables examined.

Conclusion: Platelet monocyte aggregates are useful for understanding significant differences between the mean values of different patient groups, but not useful for individual diagnosis. This result emphasizes the importance of presenting data in a clinically relevant format.

Disclosure of Interest: None Declared.

PO472-WED

A step forward in the evaluation of platelet function testing: routine, simultaneous measurement of platelet aggregation, serotonin (5-HT) content and secretion by HPLC-electrochemical detection in hemostasis laboratories

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Background: Radioisotopic 5-HT release is still the best assay to measure platelet δ -granule secretion, but has been displaced from clinical labs due to the use of radioisotopes. Lumiaggregometry is the most widely used test to assess platelet secretion (PS), but it has technical problems and high cost that limit its use.

Aims: To compare 2 PS assays: ³H-5-HT (gold standard) with HPLC-electrochemical (EC) detection assay, which allows measuring concomitantly light transmission aggregation (LTA), 5-HT secretion and 5-HT platelet content.

Methods: LTA recording is stopped with ice-cold 0.8% EDTA in saline and the platelet pellets, obtained by centrifugation, are lysed, diluted with water and internal standard and subjected to HPLC-ED testing. The secreted 5-HT is calculated by subtracting the amount measured for each agonist from the amount obtained in a control pellet (PRP+saline), which represents the platelet 100% 5-HT. For comparison, standard ³H-5-HT was performed.

Results: Sensitivity of HPLC-ED assay is 1 ng mL⁻¹. CVs were <2%. Intraclass correlation coefficient analysis showed that both assays had mean concordances ranging between 0.906–0.968 and mean agreements between 0.861–0.968 ($P < 0.0001$). Regression analysis for epinephrine showed absolute agreement for both methods. Bland Altman plots show high agreement and no evidence of systematic bias between assays. Median (percentiles 5–95%) values in paired assays ($n = 449$) in 76 subjects were 40% (0.0–63) for HPLC-ED and 42% (1.5–64) for ³5-HT test ($r = 0.87$). Mean platelet 5-HT content = 845 ± 347 ng 10⁻⁹ platelets ($n = 57$).

Conclusion: 5-HT is platelet-specific and an ideal secretion marker. HPLC-EC is highly specific, sensitive and accurate, has low CV and its overall performance is at least similar to the gold standard. Single samples permits measuring LTA and platelet 5-HT content and diagnose isolated secretion defects, with results delivered overnight. Running costs are cheap. HPLC-EC may favorably replace current PS tests. (FONDECYT 1130853).

Disclosure of Interest: None Declared.

PO473-WED

Outcomes of 65 pregnancies in 34 women with 5 different forms of inherited platelet function disorders enrolled in a retrospective and multicentric study, on behalf of eha-swg on thrombocytopenias and platelet function disorders

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Background: Pregnancy and delivery represent hemostatic challenges to women with inherited platelet function disorders (IPFD). Limited published data suggest that major bleeding is very common. However, information on outcomes and management of IPFD in pregnancy is scarce.

Aims: To improve knowledge on this matter we performed a retrospective, multicentric study aimed at systematically collecting and analyzing pregnancy outcomes in a series of women with IPFD.

Methods: 34 women with 5 different forms of IPFD confirmed by specific diagnostic criteria were enrolled: data from 65 pregnancy and 48 newborns, 9 of whom suffering from the same disease of the mother, were collected from 12 institutions around the world.

Results: Gestation was similar to that in general population, while severe bleeding events requiring blood transfusion were observed in 12.5% of deliveries. Therapeutic transfusions were administered in 50% of deliveries in women with Glanzmann thrombasthenia (GT), but in any of the women with delta storage pool disease, Hermansky-

Pudlak syndrome, P2Y₁₂ defect or defect of thromboxane A₂ receptor, who only had a few mild bleeding events. Of note, severe hemorrhages affected more than half of women with GT who received prophylactic platelet transfusions, suggesting that better preventive treatments are required in this condition. No women died of delivery-related causes or received hysterectomy to stop bleeding. Excessive bleeding at delivery requiring transfusion correlated significantly with a history of grade 3 or 4 (OR 23.4) of WHO bleeding scale and with the diagnosis (GT compared with delta storage pool disease, OR 41.6). Neither the 9 neonates who inherited the mothers' illness nor the unaffected infants experienced bleeding at birth.

Conclusion: The risk of bleeding at delivery in women with IPFD varies according to the diagnosis and can be predicted based on medical history.

Disclosure of Interest: None Declared.

PO474-WED

The value of impedance aggregometry in acute leukemia patients

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Background: Patients with acute leukemia develop abnormalities of haemostasis, leading most often to bleeding, but also to thrombotic complications, with a significant negative impact on morbidity and mortality. The prediction of these complications is difficult because of lack of laboratory markers. Although the pathogenesis of haemostatic defects is complex and multifactorial, a central role is played by the platelets.

Aims: Our study aimed to evaluate platelet function by whole blood impedance aggregometry and its possible role in bleeding and thrombosis prediction.

Methods: A number of 42 patients with newly diagnosed acute leukemia were included in the study. Platelet function testing was performed by means of multiple electrode aggregometry, using as agonists adenosine diphosphate (ADP), collagen and thrombin receptor activating peptide-6 (TRAP).

Results: Platelet function analysis showed significantly reduced aggregation response for all agonists in patients who developed hemorrhagic complications compared with those without bleeding ($P = 0.007$ on ADP, $P = 0.001$ on Collagen and $P < 0.001$ on TRAP). Moreover, platelet aggregation on TRAP stimulation showed significantly reduced response in patients with severe bleeding vs. patient with mild/moderate bleeding ($P = 0.01$). On the other hand, the patients who developed thrombotic complications displayed significant platelet hyperreactivity on all agonist compared with those without thrombosis ($P = 0.005$ on ADP, $P = 0.001$ on Collagen and $P < 0.001$ on TRAP).

Conclusion: Impedance aggregometry is a valuable tool for prediction of both hemorrhagic and thrombotic complications in acute leukemia patients. Furthermore, it may be able to predict the severity of bleeding. Wider studies are needed to confirm our observations.

Disclosure of Interest: None Declared.

PO475-WED

A national registry of patients with inherited platelet disorders: bleeding score is associated with time of follow-up

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Background: Inherited platelet deficiency and/or dysfunction may be more common in the general population than has previously been appreciated.

Aims: In order to advance our knowledge of the prevalence and clinical implications of inherited platelet disorders (IPDs) we established in 2013 the Israeli IPDs Registry.

Methods: Data on the presentation, demographics, clinical, laboratory, genetic evaluation and outcome was entered to pre-specified registration forms. The study protocol was approved by the local hospital ethics committees.

Results: To this date we have included in the registry 84 patients (male 52%) from 70 families; storage pool defects ($n = 7$), Glanzmann thrombasthenia ($n = 4$), Gray platelet syndrome ($n = 3$), Noonan syndrome ($n = 1$), Hermansky-Pudlak syndrome ($n = 3$), Jacobson syndrome ($n = 1$), MYH9-related disorder ($n = 1$), not-yet diagnosed familial thrombocytopenia ($n = 37$) and non-specific platelet function disorder ($n = 27$). Full clinical data is available for 72 (86%) patients. The median (range) age at presentation and time of follow-up were 3.64 years (1 day-17.5 years) and 9.5 (0.5-26.5) years, respectively. Nine patients (14.2%) were born to consanguineous parents. Thirty-nine patients (54%) had at least one affected family member. In 22 patients (30%) non bleeding signs and symptoms were reported including dysmorphism, mental retardation, etc. The Pediatric Bleeding Questionnaire was used to evaluate bleeding episodes; 26 (36%) were found to have a bleeding score of 2-4 and 14 (19.5%) a bleeding score of ≥ 5 . Bleeding severity was significantly associated with time of follow-up ($P = 0.024$), but not with age at diagnosis or gender.

Conclusion: Long follow-up of patients with IPDs is important as bleeding risks may increase with time. We expect that clinical and laboratory information of patients/families with IPDs gathered in a systemic format will allow for better understanding of human platelet function and better diagnosis and treatment of these patients.

Disclosure of Interest: None Declared.

PO476-WED

Plasma markers of fibrinolysis, thrombin generation and platelet activity in myeloproliferative neoplasms

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Background: Essential thrombocythemia (ET) and polycythemia vera (PV) are major subtypes of myeloproliferative neoplasms (MPN). It is

well known that MPN patients may have platelet dysfunction. However, there are few studies on the fibrinolytic system and thrombin generation in MPN.

Aims: To assess plasma-based markers of the fibrinolytic system, thrombin generation and platelet function in MPN.

Methods: We enrolled 22 patients (11 ET and 11 PV) and 10 healthy donors. Plasma levels of biomarkers related to fibrinolysis (plasminogen activator inhibitor-1 [PAI-I]), thrombin generation (prothrombin F1 + 2) and platelet activity (soluble P-selectin and soluble CD40 ligand [sCD40L]) were measured by ELISA. Platelet P2Y₁₂ function was evaluated by flow cytometry with data reported as platelet reactivity index (PRI). The results were correlated to blood counts and compared between subgroups.

Results: In MPN, levels of soluble P-selectin and sCD40L were significantly higher than normal controls. In contrast, levels of PAI-I and F1 + 2 were indistinguishable between MPN and normal controls. There was a trend towards higher plasma sCD40L in ET compared to PV ($P = 0.06$). No significant difference was found between ET and PV in levels of soluble P-selectin, PAI-I and F1 + 2. Soluble P-selectin levels were correlated to platelet and white blood counts but inversely correlated to PRI. sCD40L levels were correlated to platelet counts but inversely correlated to PRI. F1 + 2 was not correlated to blood counts or PRI, but PAI-I was correlated to platelet counts and inversely correlated to PRI.

Conclusion: Elevated plasma P-selectin and sCD40L are hallmarks of MPN, and maybe alternatives to platelet function assays. Soluble P-selectin, sCD40L and PAI were correlated to platelet counts, suggesting platelet activation and alpha granule release in MPN. The significance of such biomarkers should be investigated in large-scale studies. In contrast, plasma markers of fibrinolysis and thrombin generation are not elevated in MPN patients.

Disclosure of Interest: None Declared.

PO477-WED

Comparison of a novel automated microchip flow-chamber system and conventional platelet function tests in patients with ischemic cerebrovascular diseases

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Background: The method to assess high on-treatment platelet reactivity (HTPR) has not been established. T-TAS[®] is a novel microchip flow-chamber system to quantify platelet thrombus formation (PTF) on the collagen surface under arterial flow conditions in whole blood, and might be useful to assess HTPR.

Aims: We investigated the characteristics and usefulness of T-TAS in evaluating platelet reactivity under antiplatelet therapy by comparing with other platelet function tests.

Methods: PTF was measured in 94 patients with ischemic cerebrovascular diseases treated with clopidogrel, and/or aspirin (56 males and 38 females, age 67.7 ± 12.4 years). PTF was compared with other platelet function tests, such as platelet aggregation by light transmittance aggregometry, VerifyNow, phosphorylation of vasodilator-stimulated phosphoprotein (platelet reactivity index: PRI), platelet p-selectin expression (PS), and platelet-monocyte complex (PMC). This study was approved by the Institutional Ethics Committee of Tokyo Women's Medical University, and written informed consent was obtained from all subjects.

Results: PTF was only weakly correlated with platelet aggregation induced by adenosine diphosphate (ADP) or collagen, and PS. Concerning the influences of antiplatelet agents, the levels of PTF in patients treated with aspirin plus clopidogrel were significantly lower than those in patients treated with aspirin alone as well as clopidogrel alone. Among patients treated with clopidogrel alone, the levels of PTF, PS and PMC were significantly greater in patients with severe cerebral arterial stenosis than in patients with mild stenosis, while platelet aggregation induced by ADP, P2Y₁₂ reaction units by VerifyNow and PRI were not significantly different.

Conclusion: T-TAS can assess the effects of both aspirin and clopidogrel, and can be useful to assess platelet reactivity in patients with severe cerebral arterial stenosis.

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PO478-WED

Genetic and non-genetic determinants of MPV differ in males and females – results from the Gutenberg Health Study

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Background: Increased mean platelet volume (MPV) was associated to increased vascular morbidity and mortality. MPV is heritable as reported by large genome wide association studies. To date, a comprehensive, combined analysis including known genetic and non-genetic factors determining MPV is still lacking.

Aims: To evaluate the role of genetic and non-genetic factors for MPV variety in males and females from a large population-based cohort.

Methods: In 4,059 individuals from the Gutenberg Health Study, a sex-stratified multivariable linear regression analysis was applied to explore the associations between MPV and a number of biochemical, clinical, environmental and genetic variables.

Results: The full model including age, cardiovascular risk factors (CVRFs), laboratory parameters, medications and genetic variation explained 20.4% of females and 18.6% of males MPV variance. Oral contraceptives in females ($\beta = 0.271$, $P = 0.001$) and smoking in males ($\beta = 0.103$, $P = 0.03$) as non-genetic variables displayed the strongest associations with MPV. Nine SNPs in females and 8 SNPs in males were positively associated with MPV (β ranging from 0.412 to 0.08, $P < 0.05$). In females, interaction of rs649729 (*EDH3*) with smoking, and rs4774471 (*TPM1*) with hypertension were associated with MPV increase of 0.251 fL and 0.197 fL, respectively. In males, interaction between positive family history of myocardial infarction

and/or stroke and rs10506328 (*NFE2, COPZ1*) and/or rs2296828 (*DOCK8*) was related to MPV increase of 0.194 and 0.213 fL, respectively.

Conclusion: Our large population-based study gives evidence for a number of genetic and non-genetic factors, different for males and females, determining higher MPV. Whether the observed interactions between genetic variations, CVRFs and MPV indicates hyperactive platelets and increased risk for thrombosis remains to be further investigated by using sex-stratified methodology.

Disclosure of Interest: None Declared.

PO479-WED

Evaluation of platelets count and activation during menstrual cycle in young women in oral contraceptive use

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Background: Menstruation is a hemostatic process triggered by platelet activation, fibrin clot formation and dissolution and endometrial tissue remodeling. P-selectin is a surface glycoprotein that mediates platelet interactions among the endothelium, platelets and leukocytes, stabilizing GPIIb/IIIa-fibrinogen interaction until the formation of the platelet plug. GPIX and GPIIb/IIIa act in the adhesion and platelets aggregation processes when the blood vessel injury occurs. The interaction between platelets and leukocytes is observed in inflammatory and thrombotic processes.

Aims: To evaluate the platelet count and activation in women using oral contraceptives during menstruation cycle.

Methods: This study was approved by local Ethical Committee and consent was obtained in all cases. It included 20 healthy women (18–30 years old). The platelets evaluation was done on the 2nd day of menstruation and on the average day of the cycle. Mean fluorescence intensities of platelet glycoproteins CD41a/GPIIb/IIIa, CD61/GPIIIa, CD42a/GPIX, CD62P/P-selectin, %CD62P⁺ platelets, platelet-monocyte aggregates (PMA) and platelet-neutrophil-aggregates (PNA) were evaluated by flow cytometry. Platelets count was done by automatic method. To compare the means/medians, paired t-test and Wilcoxon test were used, respectively. $P \leq 0.05$ was considered significant.

Results: There were no differences in platelets count or platelets expression of CD41a, CD42a, %CD62P⁺ and PMA or PNA, comparing the two phases of the menstrual cycle. The average menstrual cycle day was accompanied by increased platelet expression of CD62P and CD61 ($P = 0.05$) vs. 2nd day of menstruation.

Conclusion: The platelet activation is more intense in average cycle day vs the 2nd day of menstruation, probably by the higher levels of estrogen at that time. We speculated that the MFI of CD61 and CD62P are more sensitive markers to the hormone effects and less subject to pre-analytical interferences.

Support: CNPq and FAPEMIG/Brazil.

Disclosure of Interest: None declared.

PO480-WED

Revisiting the hemostatic disorder of uremia: patients on peritoneal dialysis (CRF-PD) have normal platelet aggregation and secretion but increased platelet tissue factor activity (TF-PCA), probably the cause of intravascular clotting activation

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Background: CRF courses with accelerated atherothrombosis, major cause of death in patients under dialytic therapy. This may be explained by the accompanying inflammation, oxidative stress, endothelial damage and clotting activation.

Aims: To study the contribution of platelet TF to the systemic clotting activation in patients with terminal CRF-PD.

Methods: We studied 22 age-sex matched CRF-PD and Controls, measuring platelet aggregation (LPA), 5-HT secretion (PS), platelet P-selectin and Annexin V binding, TF-PCA (FXa nMx2*10⁻⁷ platelets), Thrombin Generation in PRP (TG-PRP) and markers of inflammation (us-CRP, TNF α), oxidative stress (AOPP), endothelial dysfunction (VWF:Ag), clotting (F1 + 2) and fibrinolysis (D-dimer)

Results: Patients had increased plasma levels of us-PCR, TNF α , IL-6, AOPP, VWF:Ag, F1 + 2 and D-dimer than controls. No differences were found in LTA, PS, P-selectin expression and Annexin V binding. Median values of platelet TF-PCA in non-stimulated platelets in CRF-PD and Controls were 44 and 20.8 nM of FXa x 2*10⁻⁷ ($P = 0.009$), but no significant differences were found in TRAP and VWF-Ristocetin activated platelets. Moreover, Velocity Index (Vi) of TG-PRP was lower in Patients than in Controls, both in non-stimulated (Median 4.5 [range 1.7–12] vs 2.5 [0.5–3.2]) and stimulated platelets (20 [11–61] vs 8.9 [1.7–42], ($P = 0.0002$ and $P = 0.0088$), respectively. TF-PCA was significantly correlated with platelet Annexin V binding ($r = 0.74$, $P = 0.008$).

Conclusion: CRF-PD courses with chronic, subclinical clotting activation. In this context, platelets circulate with increased basal TF-PCA, which raises marginally after activation. Also, TG-PRP is lower in CRF-PD than in Controls. This suggests that exposed, active TF in circulating platelets contribute to intravascular clotting activation. This could result in exhausted platelet TF under conditions of agonist demand, in line with the decreased granule content in CRF previously described (Mezzano et al. Thromb Haemost 1996)(FONDECYT 1030853).

Disclosure of Interest: None declared.

PO481-WED

Treatment of inherited platelet function disorders in children and adolescents – interdisciplinary guideline of the German Society of Thrombosis and Haemostasis research (GTH)

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Background: Although therapeutic options for prevention and treatment of bleeding due to PFD are limited, identification and classifica-

tion of PFD prior to treatment is highly recommended. The interdisciplinary S2k guideline for a rational diagnostic approach has been published elsewhere (Knöfler R. et al. *Hamostaseologie* 2014, 201-212).

Aims: Recently the German Society for Consultants and clinical disciplines (AWMF) approved the S2k guideline for treatment of inherited platelet function disorders (PFD).

Methods: The consensus expert meetings were initiated by the THROMKID study group, a quality project of the Permanent Pediatric Committee of the GTH.

Results: The underlying disease, patient's age and the clinical situation - prevention of bleeding in case of invasive procedures and control of acute bleeding - influences the choice of treatment. A therapeutic algorithm has been developed with detailed suggestions for control of local and systemic bleeding, treatment with antifibrinolytics, desmopressin (DDAVP), recombinant activated factor VII (rFVIIa) and platelet transfusions. Most forms of PFD can be treated very effectively by local treatment measures such as compression and local haemostatics combined with antifibrinolytics such as tranexamic acid (TA). The guideline also contains recommendations for the age-, body weight- and way of administration dependent dosages for various drugs including TA and DDAVP as well as rFVIIa. rFVIIa is only licensed for patients with Thrombasthenia Glanzmann and alloantibodies although it is widely used to control bleeding in patients with PFD and considered to be effective also in other receptor defects including Bernard-Soulier syndrome. Platelet concentrates may prevent and control bleeding in inherited disorders of platelet function but the risk of infection and development of alloantibodies has always to be outweighed against its transient haemostatic effect.

Conclusion: Authors consider this guideline an important tool to improve treatment of children with inherited PFD.

Disclosure of Interest: None declared.

PO482-WED

Changes in the expression of plasma and platelet micRNAs in type 2 diabetes mellitus and obesity

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Background: MicroRNAs (miRNAs) have recently gained attention as novel regulators of disease pathomechanism. Increased platelet activation is typical in type 2 diabetes mellitus (DM2) that may result in atherosclerosis and thrombotic complications. Alterations in platelet function are already initiated in case of obesity, when platelets are exposed to pre-diabetic conditions.

Aims: Here we analyzed the expression of miRNAs in plasma and platelet samples of DM2 and obese patients compared to controls to observe if cellular activation is related to abnormal miRNA levels.

Methods: We enrolled 20 DM2, 15 obese and 16 healthy individuals in this study. Platelet miRNAs were isolated from leukocyte-depleted platelets prepared by CD45 + magnetic beads (Invitrogen), while circulating miRNAs were studied in plasma samples. The quantification of selected miRNAs was performed by UPL-based RT-qPCR (Roche). The reference gene RNU43 was used for normalization. Platelet activation was analyzed via P-selectin expression, while von Willebrand factor antigen (vWFag) levels were measured to evaluate endothelial injury.

Results: In DM2 there was a significantly decreased expression of platelet miR-223 compared to normal counterparts, while in contrast, obese subjects showed increased levels. Enhanced platelet P-selectin

positivity (5.1 [3.9–8.4]%) in diabetics and in obesity (3.3 [2.6–4.6]%) showed a negative association with altered platelet miR-26b expression vs. controls (1.5 [1.1–2.4]%). Furthermore, downregulated plasma miR-424 and miR-24 in DM2 and obese subjects significantly correlated ($P = 0.014$) with elevated vWFag levels. MiR-223 content of microparticles (MPs) was also investigated, and a significant inverse relationship ($\rho = -0.428$; $P = 0.033$) was found between this miRNA level in platelets and that of MPs suggesting the release of miRNAs from platelets into MPs in DM2.

Conclusion: Altered miRNA levels may contribute to abnormal platelet and endothelial function in metabolic disorders.

Disclosure of Interest: None declared.

PO483-WED

Thrombin activated factor XI-driven platelet thrombingeneration: a new sensitive marker for hypercoagulability in stable coronary artery disease

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Background: Thrombin is essentially involved in the pathogenesis of atherothrombosis. Initially formed by inflammatory cells thrombin mediates platelet activation and platelet-dependent propagation of thrombin generation driven by factor XI-feedback activation. However, the potential of thrombin-induced platelet thrombin generation in stable coronary artery disease is not known so far.

Aims: Investigating tissue factor (TF)- and thrombin-induced platelet thrombin generation from patients with coronary artery atherosclerosis *in vitro*.

Methods: 40 patients with stable angina (without history of myocardial infarction, percutaneous coronary intervention or coronary artery bypass graft) and patients with acute non-ST-segment elevation myocardial infarction (NSTEMI) were included. For all patients > 50% stenosis in at least one coronary artery was confirmed by angiography. Age-matched healthy volunteers served as controls. Thrombin generation was induced by TF or thrombin in platelet-rich or platelet-free plasma and assessed with calibrated automated thrombography according to standardized protocols.

Results: Platelets from patients with stable angina showed significantly elevated thrombin peak induced by TF or thrombin compared to controls. However, the endogenous thrombin potential (ETP) was additionally increased in platelet-rich plasma from these patients when thrombin but not TF was used as trigger. TF as well as thrombin induced significantly elevated ETP and higher thrombin peak in platelet-rich plasma from patients with acute NSTEMI compared to controls. In contrast to platelet-rich plasma thrombin generation capacity was not significantly different in platelet-free plasma from patients with stable angina and acute NSTEMI, respectively.

Conclusion: Our data indicate that the *in vitro* capacity of platelet-based thrombin generation triggered by thrombin rather than by TF serves as a sensitive marker for platelet hypercoagulability in stable coronary artery disease.

Disclosure of Interest: None declared.

PO484-WED

Clinical and laboratory features of patients with platelet dense granule deficiency: a single institution experienceWarad D¹, Cardel L², Uhl C³, He R², Pruthi R⁴ and Chen D²¹*Pediatric Hematology Oncology, Special Coagulation Laboratory;* ²*Special Coagulation Laboratory, Mayo Clinic, Rochester, USA;* ³*Electron Microscopy Laboratory, Special Coagulation Laboratory, Mayo Clinic, Rochester, USA;* ⁴*Hematology, Special Coagulation Laboratory, Mayo Clinic, Rochester, USA*

Background: Dense granule deficiency (DGD), the most common of platelet storage-pool disorders, has heterogeneous clinical and routine laboratory features. Platelet electron microscopy (PEM) is considered the test of choice for diagnosis, however, given the complexity and high cost of PEM, a reliable pre-analytical screening mechanism is needed.

Aims: To evaluate clinical and laboratory features of individuals diagnosed with DGD by PEM.

Methods: Retrospective review.

Results: of 86 patients referred for PEM (2012–2014), 10 (12%; 4 female) were identified to have DGD. Median age at laboratory evaluation was 56.4 years (Range 10 days–71.8 years). Median platelet count was $197.5 \times 10^9/L$ (Range $27\text{--}389 \times 10^9/L$), median DG count was 0.7 ($n = 10$, 0–1.1; normal range ≥ 1.2). Two patients (20%) were asymptomatic while 8 (80%) were symptomatic: epistaxis (3/10), bruising (7/10), bleeding from superficial cuts (3/10), oral bleeds (1/10), gastrointestinal bleeding (3/10), hematuria (1/10), bleeding with tooth extraction (2/10), surgical bleeding (4/10), menorrhagia (4/4 females). Only 1 patient each had hematoma/hemarthrosis and intracranial bleed. One patient did not have documented light transmission aggregometry (LTA) while thrombocytopenia precluded testing by PFA-100 or LTA in 2/11 pt. of 8 patient with PFA-100 testing, 5/8 (62.5%) had either prolonged Col/Epi closure time 5/8 (62.5%) or Col/ADP closure time 4/8 (50%). LTA was noted to be abnormal in 4/7 (57%) and of these, 1 patient each had potential confounders with mild thrombocytopenia, mild von Willebrand factor deficiency, and atorvastatin.

Conclusion: Conventional screening tests for platelet dysfunction produce inconsistent abnormalities and have poor correlation with the clinical presentation of DGD. In our experience, most patients had mild bleeding symptoms; life-threatening bleeds are rare. PFA closure time by Col/Epi appears to be the most consistent screening abnormality even in asymptomatic patients and PEM remains indispensable for definitive diagnosis of DGD.

Disclosure of Interest: None declared.

PO485-WED

Successful use of local platelet pellet for children with severe epistaxis

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Background: Epistaxis is common bleeding site in platelet disorders. Tampon usage, platelet transfusion and/or treatment for platelet increase, fibrinolysis inhibitors are general applications for stopping of bleeding. In ITP and Glanzman disease, the continuing bleeding after classical local and systemic treatments is an important clinical problem.

Aims: We report that epistaxis was successfully stopped with local use of platelet pellet in two patient of ITP and one patient of Glanzman disease.

Methods: The preparation of platelet pellets suspension: A bag of random platelet suspension was centrifuged. Supernatant plasma was removed. Platelet sediment was resuspended with remaining plasma in own bag and withdraw a sterile injector. This suspension was dropped to the patient's nose slowly in supine position.

Results: Case 1: 12 year-old patient with acute ITP was referred to our hospital for continuing bleeding after tampon application. A new tampon with adrenalin and transamine was applied. Methyl prednisolone $30 \text{ mg kg day}^{-1}$ treatment was started. Retronasal bleeding was continued as oozing. Two erythrocyte transfusion was given because of hemoglobin declining. Case 2: 14 year-old patient with chronic ITP was admitted with epistaxis. Bleeding was continued after tampon. High dose methyl prednisolone treatment was started. Transfusion of erythrocyte for hemoglobine declining and aphaeresis platelet suspension for controlling bleeding was given next period. Case 3: 8 year-old patient with Glanzmann disease was admitted with epistaxis. Retronasal bleeding was continued after tampon usage and platelet transfusion. Epistaxis was successfully stopped with local platelet pellet dropping at these three patients.

Conclusion: Intranasal platelet pellet dropping can stop the uncontrolled epistaxis after nasal tampon use and platelet increasing treatments. In our opinion local platelet pellet dropping may be useful in nasal bleedings of other bleeding disorders such as hemophilia, aplastic anemia and leukemia.

Disclosure of Interest: None declared.

PO486-WED

Elevated soluble platelet glycoprotein vi levels in patients after living donor liver transplantationMatsumoto T^{1,2}, Takahashi N³, Usui M³, Naitoh K⁴, Wada H⁵, Ohishi K¹, Katayama N² and Isaji S⁵¹*Division of Blood Transfusion, Mie University Hospital;*²*Department of Hematology and Oncology, Mie University**Graduate School of Medicine, Tsu, Japan;* ³*Hepatobiliary**Pancreatic and Transplant Surgery, Mie University Graduate**School of Medicine, Tsu, Japan;* ⁴*Biology Laboratory, Discovery**Research, Mochida Pharmaceutical CO., Shizuoka, Japan;*⁵*Department of Molecular and Laboratory Medicine, Mie University Graduate School of Medicine, Tsu, Japan*

Background: Living donor liver transplantation (LDLT) is commonly associated with thrombocytopenia, which may be related to a poor outcome. In this study, sGPVI levels were examined in patients undergoing LDLT and the relationship between platelet activation and thrombocytopenia was evaluated in the patients who experienced a poor outcome. Upon platelet activation, the platelet surface GPVI is cleaved off by proteases releasing the soluble form of GPVI (sGPVI).

Aims: The relationship between the sGPVI levels and thrombocytopenia was examined to evaluate the mechanism of thrombocytopenia in LDLT patients with a poor outcome.

Methods: The plasma levels of sGPVI and platelet counts were determined in 79 recipients and 12 donors during LDLT. The plasma level of sGPVI was quantified using a sandwich enzyme-linked immunosorbent assay, which consisted of two mouse anti-GPVI monoclonal antibodies, F1232-7-1 and F1232-10-2 able to recognize the extracellular domain I (D1) N-terminal loop and extracellular domain D2 loop of GPVI, respectively.

Results: The platelet counts were lower in the recipients than in the donors, and those in the donors were decreased on days 1 and 3. The plasma levels of sGPVI in the recipients increased after the operation, but those in the donors increased only on day 1. The plasma levels of GPVI were higher in the recipients than in the donors on day 3. The platelet counts increased in survivors after day 14, they were higher in the survivors than in the non-survivors on days 14 and 28. Although the plasma levels of sGPVI in the non-survivors increased after the

operation, those in the survivors were high only on day 3, although they were still lower than those of the non-survivors.

Conclusion: The ratio of sGPVI to platelets of the survivors was high on days 1, 3 and 7 and low on day 28. The ratio on day 3 was higher in the non-survivors than in survivors. These findings suggest that platelet activation occurs after LDLT and it is high in the non-survivors on day 3.

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PO487-WED

Nicotinic acetylcholine receptor function in platelets from alzheimer disease patients

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Background: We have previously shown that platelets and their precursors express functional nicotinic $\alpha 7$ acetylcholine receptors (nAChR $\alpha 7$). Platelets also contain amyloid precursor protein (APP) and the enzymatic machinery for its processing. Both, nAChR $\alpha 7$ and the APP system are highly implicated in the initiation and progression of Alzheimer disease (AD). Interestingly, increased platelet reactivity is frequently reported in AD patients.

Aims: To further explore the molecular mechanisms of the platelet cholinergic system, we investigated nAChR $\alpha 7$ expression and its role in platelet function of AD patients.

Methods: Two study groups were included: AD patients (AD; $n = 16$) and age-matched healthy controls (HC; $n = 21$). We investigated platelet number, platelet degranulation and whole blood platelet aggregation. The nAChR $\alpha 7$ function was explored by the use of selective antagonists in aggregation studies and by the analysis of protein expression.

Results: Platelet number, degranulation and agonist-induced whole blood platelet aggregation were significantly increased in AD patients compared to HC. Furthermore, pre-incubation of whole blood with the nAChR $\alpha 7$ selective antagonist α -bungarotoxin led to a significantly lower aggregation response in AD-patients but not in the HC group. This is in line with our observation of a significantly higher nAChR $\alpha 7$ protein expression in AD patients compared to the HC group.

Conclusion: AD is accompanied by a prothrombotic state as indicated by increased platelet number and reactivity. Because this was accompanied by upregulation of nAChR $\alpha 7$ expression we propose a functional correlation of both, i.e. upregulation of nAChR $\alpha 7$ cause elevated platelet reactivity. The cholinergic system of platelets could serve as a peripheral model to study pathogenesis of AD.

Disclosure of Interest: None declared.

PO488-WED

Hemodiafiltration elicits an attenuated degree of platelet activation compared to hemodialysis

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Background: In patients with end stage renal disorders (ESRD) chronic hemodialysis (HD) is the most frequently applied, long-term

solution to keep ESRD patients alive. It has been shown however, that HD patients suffer from frequent cardiovascular disorders. A newer technique entitled hemodiafiltration (HDF) has been used more frequently in the treatment of patients with ESRD.

Aims: We investigated platelet and endothelial cell activation markers to compare the effects of HDF and HD.

Methods: Overall 28 patients were included in the study and citrated blood samples were obtained during the two types of treatment at 0, 1 and 4 h. Platelet P-selectin and leukocyte - platelet heterotypic aggregates were studied by flow cytometry. Soluble P- and E-selectin values were determined by ELISA, while von Willebrand factor (vWF) antigen levels were measured by immunoturbidimetry. Statistical analysis was done by the SPSS v22 software.

Results: During both types of treatment hemoconcentration occurred that was monitored by red blood cell counts and albumin values and the measured concentration values were normalized according to this effect. Platelet surface P-selectin was below 2.5% in healthy controls, but it increased substantially by 4 h, up to 8% and 14.3% in HDF and HD respectively. Monocyte-platelet heterotypic aggregates were extremely elevated by 4 h in both treatment groups, up to 69.2% in HDF and to 82.9% in HD, compared to normal values (24.6 $\pm$ 8.2%). Soluble P-selectin levels were also significantly elevated by the end of both treatments ($P < 0.001$), vWF antigen values, however, showed elevation only during HD treatment.

Conclusion: Both types of renal replacement treatments elicit platelet activation, but in HDF significantly lower values were obtained. Endothelial activation could also only be detected during HD treatment. The attenuated effects of HDF compared to HD may contribute to a favorable cardiovascular outcome in this treatment modality.

Disclosure of Interest: None declared.

PO489-WED

Twice-daily dosing of ASA overcomes reduced platelet response to once-daily ASA after coronary artery bypass graft surgery: a randomized controlled trial

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Background: Low-dose ASA (81 mg daily) is administered after coronary artery bypass graft surgery (CABG) to prevent graft failure. ASA hyporesponsiveness, defined as a failure to fully suppress thromboxane (TX) production, occurs in the immediate postoperative period and may compromise the effectiveness of ASA. In a recent randomized trial, we tested the hypothesis that resistance to low-dose ASA after CABG is caused by increased platelet turnover, and thus, is overcome by more frequent (four-times daily) ASA dosing. Although the four-times daily regimen successfully reduces ASA resistance in the postoperative period, this regimen is inconvenient for patients and health care providers.

Aims: We performed a randomized trial to determine whether a simpler twice-daily ASA dosing regimen overcomes the resistance observed with once daily-dosing.

Methods: Adults undergoing CABG with or without valve surgery were randomized to: 81 mg once-daily, 325 mg once-daily, or 162 mg twice-daily. ASA was started on post-operative day 1 and continued until day 7 or hospital discharge. Serum TXB₂ levels were measured

daily before the first dose of daily ASA. The primary outcome was the median TXB₂ level on postoperative day 4.

Results: A total of 68 patients (mean age 65, females 12%) were randomized. On postoperative day 4, median TXB₂ levels in the group receiving ASA 81 mg once-daily was 4.2 ng mL⁻¹ (Q1, Q3; 1.5, 7.5 ng mL⁻¹). The median TXB₂ levels were significantly lower in the group randomized to ASA 162 mg twice-daily 1.1 ng mL⁻¹ (Q1, Q3; 0.7, 2.7 ng mL⁻¹) and non-significantly lower in the group randomized to ASA 325 mg once-daily 1.9 ng mL⁻¹ (Q1, Q3; 0.9, 4.7 ng mL⁻¹), $P = 0.02$ and $P = 0.12$, respectively.

Conclusion: ASA hyporesponsiveness following CABG is overcome by twice-daily ASA administration. Twice-daily ASA administration post CABG provides a novel and convenient treatment regimen to reduce ASA hyporesponsiveness. A trial powered for relevant clinical outcomes is needed to assess the potential clinical benefit of the twice-daily regimen.

Disclosure of Interest: None declared.

PO490-WED

Increased mitochondrial electron transport associated with apoptosis in hyper-reactive platelets of patients with acute pulmonary embolism

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Background: Acute pulmonary embolism (PE) can cause shear stress and hemolysis, the latter of which can cause oxidative injury.

Aims: This work tests the hypothesis that physiological stresses from acute intermediate risk PE stimulate platelet mitochondria, leading to an apoptotic state that causes platelet hyperactivation.

Methods: Patients ($n = 30$) had image-proven PE, SBP > 90 mmHg and right ventricular (RV) dysfunction by echocardiogram or elevated biomarkers; controls ($n = 8$) were healthy volunteers. Blood for platelet respiration (EDTA) was obtained at diagnosis (or first sample) and 24 h later. Whole platelet O₂ consumption was measured in buffer containing glucose+octanoate with oligomycin to block ATP synthase (LEAK state) and subsequently in the presence of the protonophore FCCP, titrated to assess maximal electron transport (ETS state). Extramitochondrial oxygen consumption was measured by adding rotenone and antimycin-A. Platelet lysates (40 µg) were resolved by gel electrophoresis. Bcl-2 and cytosolic cytochrome C were detected by western blot and quantified with densitometry.

Results: At diagnosis, intact platelets from PE patients had significantly elevated ETS state O₂ consumption vs. controls (47 ± 4.3 vs. 32 ± 2.1 pmol*s⁻¹/10⁶ cells, $P = 0.002$) and in LEAK (5.1 ± 0.63 vs. 3.7 ± 0.38 , $P = 0.04$) with no measurable extramitochondrial O₂ consumption. By 24 h, all platelet respiratory parameters decreased to levels not different from control patients ($P > 0.05$). These changes were accompanied by significant decrease in mitochondrial Bcl-2 protein levels, together with increase in cytosolic cytochrome C in PE patients ($P < 0.05$ vs. controls). On thromboelastography, unstimulated maximum amplitude was increased with PE, 23 ± 18.3 vs. 8 ± 5.9 mm ($P = 0.003$).

Conclusion: Patients with PE and RV dysfunction have circulating platelets with pre-apoptotic mitochondria coincident with platelet hyper-coagulability on TEG, a phenotype that could adversely affect blood rheology in acute PE.

Disclosure of Interest: None declared.

PO491-WED

Antenatal genetic analysis of a Japanese type I CD36-deficient woman in the pregnancy

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Background: CD36 is a glycoprotein expressed in various human tissues, including platelets, monocytes, erythroblasts. Type I CD36-deficient individuals (deficient in platelets and monocytes) can be immunized to CD36 through pregnancy or blood transfusion and develop the anti-CD36 isoantibody (also known as anti-Nak^a). Thus neonatal alloimmune thrombocytopenia (NAIT) has been reported to be caused by the anti-CD36 antibody. In this study, we performed analyses of CD36 from a Japanese pregnant woman and her family.

Aims: A 30-year-old Japanese woman in the pregnancy had no history of blood transfusion. Before the pregnancy, she donated blood and a recipient was transfused with her platelets developed TRALI. After screening tests for anti-platelet antibodies, the high titer anti-CD36 antibody ($\times 32768$) was detected from her plasma.

Methods: A patient and her family blood samples were collected after the written informed consent has been obtained. We extracted genomic DNA, amplified the all exons of CD36 by PCR using allele-specific primers, and analyzed DNA sequences.

Results: She was confirmed as having type I CD36 deficiency, since her platelets and monocytes both lacked CD36 expression by FCM. Direct sequencing analyses showed she was homozygous, and her parents were heterozygous for a T111SfsX22 (c.329_330delAC) mutation in exon5. Gene mutation of CD36 was not found in her husband, therefore, the fetus expected to be CD36 positive. In the 38th gestational week, she delivered a boy by an urgent cesarean section. Newborn platelet count was slightly decreased in 7.1×10^9 /L after birth, he received immunoglobulin therapy and was transfused with the washing CD36 negative platelets suspension donated from his mother. The platelets increased to 81×10^9 /L. Anti-CD36 antibody was $\times 1024$ but gradually decreased before discharge in day 14.

Conclusion: Premature diagnosis is necessary for NAIT and our experience suggested CD36 mutation analysis was useful for understanding the pathogenesis of anti-CD36 antibody induces NAIT.

Disclosure of Interest: None declared.

PO492-WED

A reliable and simple diagnostic approach to detect platelet secretion defects in subjects with mucocutaneous bleeding

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Background: Mild platelet function defects (PFDs) are complex, difficult to diagnose and require extensive platelet function testing on fresh blood samples, so their prevalence may be underestimated. Secretion defects are among the most common PFDs.

Aims: To assess the diagnostic value of a simple assay for platelet markers of dense (CD63) and alpha granule (P-selectin) secretion that can be performed on blood taken locally and then sent to a central laboratory for analysis.

Methods: Subjects with impaired dense granule secretion ($n = 9$) were identified within the GAPP (ISRCTN 77951167) study population ($n = 61$) using lumiaggregometry (LA). Platelet CD63 and P-selectin were assessed in unstimulated and stimulated (ADP/U46619, TRAP-6 and AA/epinephrine) whole blood samples that were stabilized with PAMFix (Platelet Solutions Ltd., UK) and analysed using flow cytometry (FC) within 9 days. Normal ranges (5–95th percentiles) were established using healthy volunteers ($n = 41$).

Results: All 9 patients with reduced secretion on LA presented with abnormal results on the FC assay. Two had a very high level of CD63 even in the unstimulated samples with reduced P-selectin, which is characteristic of Hermansky-Pudlak syndrome type 2. Four subjects with the lowest levels of secretion on LA had reduced CD63 with all three stimulants and reduced P-selectin with at least one stimulant; this pattern was not observed in subjects with other types of platelet defects ($n = 24$). In another 3 subjects both CD63 and P-selectin were below the normal range with at least one agonist. This pattern of results was seen in 6 of 24 subjects who were labelled as mildly abnormal by LA. Only two subject with normal platelet function as judged by LA ($n = 28$) had CD63 expression slightly below the normal range and P-selectin within the normal range.

Conclusion: Abnormal granule secretion can be identified using a new simple assay that could be performed remotely as first line diagnostic testing for PFDs.

Disclosure of Interest: N. Dovlatova Employee of: Platelet Solutions Ltd., M. Lordkipanidzé: None declared, G. Lowe: None declared, B. Dawood: None declared, J. May Shareholder of: Platelet Solutions Ltd., S. Heptinstall Shareholder of: Platelet Solutions Ltd., S. Watson: None declared, S. Fox Shareholder of: Platelet Solutions Ltd.

PO493-WED

Platelet CD40L controls bleeding during P2Y12 blockers treatment

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Background: In patients with Acute Coronary Syndrome (ACS), P2Y12 blockers are associated with increased bleeding risk significantly reducing treatment benefits. CD40 Ligand (CD40L) is modulated by P2Y12 blockers. CD40L deficient mice exhibit thrombus instability and increased bleeding.

Aims: It is unknown whether platelet CD40L and P2Y12 inhibition are associated with bleeding events through independent or interactive effects. We investigated the association between platelet CD40L, ADP signaling and bleeding occurrence in 244 patients receiving thienopyridines. We further evaluated whether platelet-associated CD40L take part in bleeding control in mice treated with ticagrelor.

Methods: Platelet CD40L surface expression (pCD40L) before and after PAR-1 stimulation was quantified. Results were compared to the platelet reactivity index, (PRI-VASP). Tail bleeding time was assayed in mice treated with ticagrelor and transfused with either CD40L^{-/-} or CD40L^{+/+} platelets.

Results: pCD40L was reduced under thienopyridine therapies. In multivariate analysis basal pCD40L independently contributes to the bleeding risk. *In-vitro* ADP degradation and P2Y12 inhibition partially reduced the TRAP-induced pCD40L and totally blunted CD40L release. Ticagrelor-treated mice exhibit a 2.5 fold increase tail bleeding time as compared to control animals. Infusion of CD40L^{+/+} platelets significantly reduces bleeding time prolongation whereas CD40L^{-/-} platelets fail to.

Conclusion: Blocking the ADP pathway both *in-vitro* and *in-vivo* reduces pCD40L surface expression. In thienopyridine treated patients, pCD40L independently associates with the bleeding risk. Experiments performed in mice indicate the involvement of pCD40L expression in the increased bleeding tendency induced by P2Y12 blockers. pCD40L may represent a novel tool to assess bleeding risk in ACS patients treated with P2Y12 blockers.

Disclosure of Interest: None declared.

PO494-WED

Clinical and genetic predictors of poor platelet response to clopidogrel in patients who underwent percutaneous coronary intervention

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Background: Platelet response to the antiplatelet prodrug clopidogrel varies among patients and a poor response has been linked to ischemic events.

Aims: To investigate clinical and genetic predictors of platelet response to clopidogrel.

Methods: ASCLOGEN is an Institutional Review Board approved study which enrolled patients who underwent percutaneous coronary intervention (PCI). Platelet response to clopidogrel was measured using light transmission aggregometry (LTA), VerifyNow[®] P2Y12, and VASP. Multiple imputation (MI) chained equation approach with random forest imputation per variable and MI weighted elastic net method (MI-WENet) were used to select clinical predictors of clopidogrel response. Linear regression models of response measures were fit with the chosen predictors to the imputed datasets. A genome-wide association study (GWAS) was performed using a cardiovascular gene-centric Illumina 50 K SNP array. Variants were filtered for MAF, H-W equilibrium, and call rate. Per-SNP GWAS regressions included the non-genetic predictors, sex, age and 2 variables for genetic relatedness. A P -value $< 2.29E-6$ was deemed significant for single variant tests.

Results: After quality control, 423 patients were eligible. Sixty-five multiply imputed variables were obtained and considered as predictors for clopidogrel response. The MI-WENet method selected type 2 diabetes (Betas: 6.16–28.87, P -values: ≤ 0.001 –0.01), proton pump inhibitor (PPI) intake (Betas: 4.46–34.13, P -values: ≤ 0.001), and statin non-use (Betas: 5.87–22.48, P -values: ≤ 0.001 –0.01) as predictors of poor clopidogrel response. Conditional on these predictors, GWAS found only 1 genome-wide SNP: rs4244285, *CYP2C19**2 variant (Betas: 6.3–40.34, P -values: < 0.001).

Conclusion: Type 2 diabetes, PPI intake, statin non-use, and *CYP2C19**2 genetic variant independently predict poor platelet response to clopidogrel.

Disclosure of Interest: None declared.

PO495-WED

No increase in platelet aggregation in healthy women starting combined oral contraceptives

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Background: An increased risk of venous thrombosis in users of combined oral contraceptives (COCs) is generally recognised, and changes in the coagulation system in COC users have been investigated extensively. However, only a few and small early studies assessed platelet aggregation in COC users.

Aims: To determine whether COC treatment influences platelet aggregation in healthy women.

Methods: We included 18 healthy women between 18 and 34 years who wanted to start COC treatment. Exclusion criteria were pregnancy, childbirth or use of systemic hormonal therapy within the last 3 months, systemic inflammatory disease, and known coagulation disorders. The COCs used contained 35 µg ethinyl estradiol and 150 mg levonorgestrel. Platelet-inhibiting medications (aspirin, ADP-inhibitors, NSAIDs, fish oil) were not allowed within seven days before blood sampling. Blood samples were obtained before COC start and after three and six COC cycles. Platelet aggregation was measured using whole-blood impedance aggregometry. Blood was sampled in hirudine tubes. Platelets were stimulated with ADP 6.5 µM, collagen 3.2 µL mL⁻¹, and ristocetin 0.77 mg mL⁻¹ (standard test concentration). Outcomes were changes in total aggregation expressed as AUC after start on COCs. The study was approved by The Central Denmark Region Committees on Health Research Ethics, and informed consent was obtained.

Results: Sixteen women completed all three samples. For all participants, platelet aggregation was within the reference interval. Baseline aggregation values (mean [95%CI]) were for ADP, 821 [768–874] AUC, for collagen, 696 [630–762] AUC, and for ristocetin, 858 [721–1048] AUC. We found no change in platelet aggregation with ADP ($P = 0.48$), collagen ($P = 0.84$), or ristocetin ($P = 0.67$) after 6 months of COC use when analysed by one-way ANOVA.

Conclusion: The present study demonstrated no change in platelet aggregation after start on COCs in healthy women.

Disclosure of Interest: None declared.

PO496-WED

The platelets characteristics in type 2 diabetes mellitus (T2DM)Buryachkovskaya L, Uchitel IA, Sumarokov AB and Docenko UV
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Background: It is well established that type 2 diabetes mellitus (T2DM) is associated with an increased risk of thrombosis and vascular events. Platelets play a crucial role in the pathogenesis of thrombotic complications during T2DM

Aims: The aim of our study was to reveal phenotypic platelets features which can be capable for thrombosis formation in T2DM pts

Methods: There were included 28 pts with stable coronary artery disease (CAD) and T2DM, 36 CAD pts and 24 healthy subjects (HS). Spontaneous and 1.0 and 5.0 µM ADP-induced aggregation was determined using an aggregation analyzer BIOLA (Russia). All patients did not receive any antiplatelet therapy for at least two weeks before platelets investigation. Platelet morphology, leukocyte-platelet aggregates (LPA) and erythrocyte-platelet aggregates (EPA) were assessed in whole blood by scanning electron microscopy. MPV was measured using automatic blood counter

Results: In all CAD pts with T2DM the number of large platelets which are characterized by increased expression of glycoprotein receptors and metabolism was elevated ($2.8 \pm 1.7\%$ vs $1.3 \pm 1.1\%$ in CAD and 0% in HS). It was also related to MPV increase (12.4 ± 1.7 in

T2DM pts vs 9.6 ± 1.1 in CAD pts and 7.6 ± 1.1 in HS). The level of activated platelets with pseudopodium was also elevated ($46.8 \pm 5.2\%$ vs $34.6 \pm 2.4\%$ in CAD and 4.2% in HS). Spontaneous aggregation was detected in 81% T2DM pts vs 34% CAD pts and was absent in HS. ADP-induced aggregation was increased in 72.4% T2DM pts vs 36% CAD pts. Up to 25% of red blood cells from T2DM pts were strained or echinocyte. All such pts revealed red blood cells fragments ($3.4 \pm 1.0\%$). Such disorders were absent in CAD pts and HS. EPA were detected in all pts with T2DM and only in 28.6% CAD pts. From 11.2% to 60% leukocytes were concentrated in LPA in all pts. In CAD pts LPA were revealed only in 28.6% pts in whose who were also EPA positive

Conclusion: The detected changes of blood cells could be the reason of the increased risk of thrombosis and rheology changes in T2DM pts

Disclosure of Interest: None declared.

PO497-WED

Study of platelet function in hemophilia a in the region of murcia

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Background: Haemophilia A (HA) is considered a classical secondary haemostatic defect. Treatment of HA is therapy of FVIII. FVIII interacts with platelets, but limited data on platelet procoagulant activity are available in HA.

Aims: Our aim was to characterize platelet function in 11 patients (age between 6–65 years) with haemophilia A severe ($< 1\%$ FVIII) without inhibitor, before and after 15 min FVIII, and 12 healthy volunteers (age around 28 years).

Methods: All of them have a normal count of platelets ($150\text{--}450 \times 10^3/\text{microL}$) and nobody had taken nonsteroidal anti-inflammatory. We determine the amount of factor FVIII by chromogenic method. For study of optical aggregation we use PPP and PRP. We follow the recommendations for the standardization of light transmission aggregometry of subcommittee of SCC/ISTH. We analyze platelet responsiveness with different agonists (ADP, COL, AA, EPI, RISTO and TRAP). Platelet aggregation was also measured by flow cytometry (BD FACSCalibur) with CD-61-PerCP using TRAP as agonist. Likewise, we determine the expression of P-Selectina (CD62) through the mean fluorescence intensity.

Results: The results are similar between controls and patients and between patients after and before FVIII. The results are shown in the following table.

	Average of patients basal	Average of patients after FVIII	Average of control
FVIII (U dL ⁻¹)	3	94.8	101
ADP (%)	56	54	53
COL (%)	74	78	75
TRAP (%)	49	39	47
ASPI (%)	84	86	84
RISTO (%)	80	87	88
EPI (%)	58	60	67
Aggregation cytometric	92.7	91.6	92.6
CD62	27.8	24.35	26.8

Conclusion: In this study platelet function is similar between controls and patients and between patients after and before FVIII. More studies are needed to standardize both techniques and correlate results.

Disclosure of Interest: None declared.

PO498-WED

Reduced platelet activity under induced oxidative stress conditions in gilbert's syndrome

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Background: Individuals with Gilbert's syndrome (GS) expressing mild unconjugated hyperbilirubinemia (UCB) and increased circulating antioxidants (AOX), have a reduced risk of cardiovascular disease (CVD). Oxidative stress induced platelet activation is central to the risk of developing thrombosis and CVD, in pre-thrombotic conditions such as diabetes and obesity. Severe acute exercise in sedentary individuals induces a significant oxidative stress, increasing platelet activation and aggregation, which is related to processes underpinning thrombosis and CVD

Aims: Here, we evaluate if elevated levels of UCB in GS can attenuate thrombotic risk factors in a model of exercise induced oxidative stress.

Methods: Blood samples, pre and post continuous aerobic exercise on a cycle ergometer (1 h at 70% VO₂Max) were collected from matched GS and control subjects. Platelet surface marker expression of CD42, PAC-1 and CD62P was assessed using flow cytometry. Platelet aggregation using (ADP, collagen and arachidonic acid (AA)) and biochemistry profile were also evaluated.

Results: A significant reduction in the expression of platelet surface marker P-selectin, along with reduced collagen and AA induced platelet aggregation existed in GS vs. control, pre and post exercise ($P < 0.05$ all variables). Increased levels of UCB, HDL along with reduced levels of CRP and LDL pre exercise were found in GS when compared with control ($P < 0.05$ all variables)

Conclusion: The results from this study indicate possible mechanisms whereby elevated bilirubin neutralizes platelet activation under OS conditions in GS. Reduced P-selectin expression in addition to decreased AA and collagen induced platelet aggregation pre and post exercise signifies the potential of UCB related AOX capacity in inhibiting platelet activation, aggregation and granule release. Reduced platelet activity, inflammation and improved lipid profile can significantly reduce the CVD risk in GS when compared with normal population under resting and OS conditions.

Disclosure of Interest: None declared.

Platelet disorders, inherited / acquired III

PO499-WED

Cerebral venous thrombosis occurring during oxymetholone therapy in a patient with fanconi aplastic anemia

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Background: Oxymetholone, an androgen analogue, is widely used in the treatment of Fanconi aplastic anemia (FAA). Androgen is known to cause cerebral venous thrombosis (CVT).

Aims: To attract attention thrombotic risk of oxymetholone treatment in patients with FAA. Venous thrombosis may develop and the complaint of the patient can similar to intracranial hemorrhage

Methods: This case report point to the thrombotic risk of oxymetholone in FAA patients.

Results: A 9-year-old girl under monitoring with a diagnosis of FAA presented to our hospital with severe headache. She had been receiving oxymetholone therapy for 1 year. Neurological examination was normal. Superior sagittal sinus thrombosis was detected at magnetic reso-

nance imaging (MRI) of the brain. The thrombosis was thought to be oxymetholone-related, and oxymetholone therapy was discontinued. The patient was started on enoxaparin therapy and the symptoms resolved after 3 months following to enoxaparin treatment.. Control images were normal 4 months later .

Conclusion: We think that the risk of venous thrombosis must be considered with administering oxymetholone therapy in patients with FAA who has acute headache complaint in addition to intracranial hemorrhage due to thrombocytopenia.

Disclosure of Interest: None declared.

PO500-WED

Management of oral anticoagulant therapy interruption in childrenNewall F^{1,2,3,4}, Kommidi V⁵ and Monagle P^{1,3,5}

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Background: There are no robust recommendations relating to the management of anticoagulant therapy interruption in children requiring oral anticoagulant therapy.

Aims: This study sought to identify the clinical outcomes associated with current bridging practices employed at a major metropolitan children's hospital for children requiring warfarin therapy.

Methods: Participants were selected from the warfarin management registry of the Clinical Haematology service between January 2004 and December 2013. The admission history of these patients was queried to identify admissions where anticoagulant therapy would typically be interrupted: dental extraction, cerebral angiography or cardiac angiography/catheterization. Data relating to demographics, anticoagulant therapy interruption +/- bridging and clinical outcomes were recorded.

Results: A total of 61 admissions for children aged between 1 year and 17 years and 11 months were analysed (56% male; mean age 10+/- 4.2 years). Congenital heart disease was the primary underlying disease. Cardiac catheterisation was most common reason for anticoagulant therapy interruption (74%), followed by cerebral angiography (20%) and dental extractions (6%). Warfarin was ceased during all but one of admissions and was withheld for a median time of 4 (1-9) days prior to the procedure. Anticoagulant therapy interruption for children with Moyamoya disease was more consistently managed compared to other underlying disease groups. There were no instances of major bleeding ($n = 0$) or thrombotic events ($n = 0$)

Conclusion: This study described the current practices and outcomes associated with bridging therapy at one institution thereby filling a gap in the paediatric bridging knowledge base. It achieved this by analysing the largest and most representative cohort to date. This project is a stepping stone from which future studies of safety and efficacy of paediatric bridging anticoagulation can be developed

Disclosure of Interest: None declared.

PO501-WED

Thrombosis in children with systemic lupus erythematosus: endogenous and exogenous risk factors

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Background: Thrombosis in children with systemic lupus erythematosus (SLE) can be resulted from genetic predisposition, vascular endothelial injury due to autoimmune inflammation or presence of antiphospholipid antibodies (APA), plasminogen activator inhibitor-1 (PAI-I) gene mutation that inhibits of fibrinolysis.

Aims: To clarify how the most common gene mutation and APA influence the course of SLE.

Methods: 24 children with SLE and thrombotic events (age 6–18 years) were examined. The duration of the disease – one month to 5 years. Factor V Leiden (FV), prothrombin G20210A, methylenetetrahydrofolate reductase (MTHFR), methionine synthase reductase (MTRR), methionine synthase (MTR), PAI-I gene mutation were investigated. The levels of APA (anticardiolipin antibodies IgG, IgM and lupus anticoagulant), protein C were measured.

Results: Homozygous for PAI-I 4G/4G were 11 (45.8%) patients. All 24 (100%) children were heterozygous for at least one of the enzymes MTHFR, MTRR or MTR. Only 2 (8.3%) girls were homo- and heterozygous for FV. Nobody had prothrombin gene mutation. Fourteen (58.8%) patients were positive for APA. Nobody had protein C deficiency. Thrombosis more often occurred in disease onset or in exacerbation of SLE. The serious thrombotic events were diagnosed in 7 (29.1%) patients. Four children presents with stroke and hemiparesis in disease onset. Three patients with stroke were homozygous for PAI-I 4G/4G and heterozygous for MTHFR, MTRR, MTR, one girl with stroke had gene mutations of enzymes and antiphospholipid syndrome (APS). Two children had thrombosis of internal organs: renal vein thrombosis and thrombosis of hepatic vein, both homozygous for PAI-I 4G/4G. Girl with renal vein thrombosis had also APS. One girl homozygous for PAI-I 4G/4G and APS developed aseptic necrosis of the femoral head. Other 4 homozygous for PAI-I 4G/4G had thrombovasculitis of cutaneous vessels.

Conclusion: Both inherited and acquired factors can lead to the most severe thrombotic complications.

Disclosure of Interest: None declared.

PO502-WED

Evaluation of age-dependent dosing recommendations for the administration of tinzaparin in the treatment of paediatric thrombosis

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Background: Current dosing recommendations for off-label use of tinzaparin in children are based on un-validated data from a single, small study.

Aims: This study aimed to determine whether age-related tinzaparin dosing recommendations are successful in achieving therapeutic anticoagulation in children treated for thrombosis.

Methods: Data were collected retrospectively over a 6-year period, 2008 - 2014, for children treated with tinzaparin. The recommended initial dose for those aged 0- < 2 months was 275 U kg⁻¹; 2-

< 12 months, 250 U kg⁻¹; 1- < 5 yrs, 240 U kg⁻¹; 5- < 10 years, 200 U kg⁻¹; 10-16 years, 175 U kg⁻¹. An anti-factor Xa activity level of 0.5-1.0 IU mL⁻¹ at 3 h post dose was considered therapeutic. Details of haemorrhage, recurrent thrombosis and post-thrombotic syndrome (PTS) were recorded.

Results: 30 children were studied, 15 males, median age 63 months. Site of thrombosis was: lower limb deep vein thrombosis (DVT), 15; upper limb DVT, 3; pulmonary embolism, 3; cerebral sinus thrombosis, 6; other, 5. The median daily dose of tinzaparin required to achieve therapeutic anticoagulation was: 275 U kg⁻¹ (range 210-275 U kg⁻¹) in children aged 0- < 2 months (*n* = 6); 270 U kg⁻¹ (range 75-402 U kg⁻¹) aged 2- < 12 months (*n* = 6); 228 U kg⁻¹ (range 214-243 U kg⁻¹) aged 1- < 5 yrs (*n* = 3); 200 U kg⁻¹ (range 169-316 U kg⁻¹) aged 5- < 10 years (*n* = 8) and 178 U kg⁻¹ (range 173-375 U kg⁻¹) in those aged 10-16 years (*n* = 7). For children receiving recommended doses, median time to achieve therapeutic anticoagulation was 2.5 days (range 1 - 16). Tinzaparin was continued for a median of 3 months and 2 adolescents were converted to warfarin for long term therapy. Complications included: death, 0; major haemorrhage, 0; recurrent thrombosis, 1; PTS, 3.

Conclusion: Tinzaparin is safe and effective treatment for paediatric thrombosis. These data support the validity of current age-dependent dosing recommendations. However, significant inter-individual variability means that intensity of anticoagulation should continue to be monitored using anti-factor Xa activity levels.

Disclosure of Interest: None declared.

PO503-WED

Using a functional platelet activity analysis in children with unexplained bleeding. first results from russia

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Background: Detecting several glycoprotein deficiencies on platelet (plt) surface is known to confirm the diagnosis of inherited platelet disorder (IPD).

Aims: To assess the role of a modification of platelet flow cytometry (PFC) method-functional platelet activity analysis (FPAA) in children with unexplained bleeding.

Methods: 13 pts (2-14 yo) with mild to moderate mucocutaneous bleeding (*n* = 9), menorrhagia (*n* = 2), spontaneous subdural hematoma (*n* = 1) or isolated oropharyngeal bleeding (*n* = 1) without a known history of coagulopathy were included. Whole blood plt were studied by FC either in native or activated with collagen-related peptide (0.18 mkg mL⁻¹) and thrombin receptor activating peptide (12.5 mkM). Fluorescently labeled antibodies against CD42b, CD61 and PAC-1, and CD62p were used; dense granule (DG) release was studied using loading with mepacrine.

Results: After standard CF had been performed, all but two pts displayed at least one defect. Isolated altered mepacrine uptake (AMU) have been evaluated in 4, lowered PAC1-in 1, different combined alterations (AMU+PAC1, PAC1 + CD42b, PAC1 + CD62p)-in 6 children. After the activation, 6 pts with AMU (*n* = 3), lowered PAC1 and CD42b (*n* = 1), or no defect (*n* = 2) appeared to have normal plt responses. Isolated DG deficiency was confirmed in 4 pts. In 3 of them with previously diagnosed PAC1 deficiency, defect of this pathway was not confirmed. Additional plt alterations were evaluated in 2 pts. One with previously reported combined PAC1 + CD62p deficiency had impaired mepacrine output, while no other alterations were

detected. FPAA led to rule out initial alterations in 6 (46.15%) pts, while new functional defects were discovered in 2 (16.7%) children. No one with previously recognized PAC1 and CD62p deficiency had these alterations after plt activation. DG deficiency was proved in 4 of 8 pts, who had initial AMU.

Conclusion: A part of functional defects could be over-/misdiagnosed, using a standard method of PCF. Further research is needed to investigate the role of FPAA in IPD.

Disclosure of Interest: None declared.

PO504-WED

Four young children with thrombotic complications as a result of antithrombin heparin-binding defects

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Background: Antithrombin deficiency is a risk factor for thrombotic events. Type II heparin-binding defects may be particularly difficult to identify, especially during or immediately after an acute thrombotic event. We report thrombotic events in 4 young children, the result of Antithrombin heparin-binding defects.

Aims: We undertook molecular genetic analysis of the *SERPINC1* gene to investigate a possible diagnosis of Antithrombin deficiency. Case 1 was a preterm new born with a dusky pale right forearm and ischaemia of the right hand, the result of a right brachial artery thrombus following a failed venepuncture attempt. Case 2 was an 11 month old girl who developed an ischaemic stroke following cardiac surgery. The third was a 3 month old girl noted to have a right sided hemiplegia and MCA infarct. The fourth patient was 17 months old with ischaemic cardiomyopathy and acute occlusion of the right coronary artery. Higher doses of heparin were required to attain therapeutic levels.

Methods: Analysis of the *SERPINC1* gene by PCR amplification and direct sequencing was performed on all affected children. Sequencing was performed on an ABI 3130xl analyser. Sequences were analysed using Mutation Surveyor software and compared against the Ensembl reference sequence (ENST00000367698).

Results: Three of the affected children were shown to be heterozygous for a c.218C>T substitution and the fourth a for a c.166C>T substitution, all in exon 2 of *SERPINC1*. The former results in a Proline to Leucine (p.Pro73Leu) mutation at codon 73 and the latter an Arginine to Cysteine mutation at codon 56 (p.Arg56Cys), both are known Antithrombin heparin-binding defect mutations. Only one parent was known to be symptomatic.

Conclusion: Heparin-binding defects of Antithrombin are not only risk factors for thrombotic events but can also interfere with standard antithrombotic therapeutic regimen. Knowledge of the presence of a heparin-binding defect can not only explain a thrombotic phenotype but can also help target treatment appropriately.

Disclosure of Interest: None declared.

PO505-WED

Risk of thrombosis in a cohort of pediatric acute lymphoblastic leukemia patients

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Background: Acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood with increased risk of thrombosis

related to the use of steroid, L-asparaginase and placement of catheters, in addition to the malignancy itself.

Aims: Our knowledge on the the incidence and risk factors that may increase the risk of thrombotic events among patients with pediatric ALL is limited.

Methods: A total of 164 patients with pediatric ALL who were diagnosed between January 2008 and January 2015 and received St Jude Total XV treatment protocol with placement of port-catheters were included. of these patients 11 (6.7%) developed thrombotic events.

Results: The median age of the diagnosis of ALL among the patients who developed thrombosis were 9 years (3–19); 7 (64%) were males. The median WBC at diagnosis was $20.8 \times 10^9/L$ (2.4–166) and 9 (82%) were CALLA+ B-cell ALL. The sites of thrombosis were snovenous thrombosis in 6 (55%), popliteal vein ($n = 1$), jugular vein ($n = 1$), basilic vein ($n = 1$), femoral vein and associated pulmonary thromboembolism (PTE) ($n = 1$), MCA ($n = 1$). The median time from diagnosis of ALL to thrombotic event was 2 months (1–36 months). One pateint who developed femoral vein thrombosis and PTE had recurrence of PTE under enoxaparine. Of the patients who developed thrombotic events, none had plasma protein C, protein S and anti-thrombin III deficiencies. Factor V G1691A was heterozygous in 3 (27%), Prothrombin G201210A was heterozygous in 1 (9%) and MTHFR 677 was in homozygous state in 2 (18%) of the patients with normal homocysteine levels. All of the patients received anticoagulation with enoxaparine, except for the patient with PTE. None of the patients with cerebrovascular thrombosis developed sequela, except for the patient with MCA infarct, who transformed to hemorrhagic infarct.

Conclusion: The risk of thrombotic events and their consequences add additional morbidities and potential mortalities to the course of pediatric ALL treatment.

Disclosure of Interest: None declared.

PO506-WED

Comparison of the impact of *serpinc1*, *proc* and *prosr1* mutations on thrombotic phenotypes in children and adults: an observational multicenter cohort study

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Background: Venous thromboembolism [TE] is a multifactorial disease and antithrombin [ATD], protein C [PCD] or protein S [PSD] deficiency constitutes a major risk factor.

Aims: To compare clinical TE phenotypes among children and adults.

Methods: Since 07-1996 a comprehensive thrombophilia screening was performed in 688 TE patients (children[C]: 0.1 to < 18 years [$n = 367$]; adults[A]: 18 to < 55 years [$n = 321$]). Along with plasma-based coagulation assays ATD, PCD and PSD were confirmed by molecular diagnosis. To compare the rates of deficiency phenotypes, TE-loc-

tions, spontaneous vs. provoked TE, family history, descriptive analysis, non-parametric statistics and Chi-square or Fisher's exact test was applied. In addition logistic regression was performed to calculate the odds/95% CIs for recurrent TE.

Results: 6.5% of patients carried ATD, 5.7% PCD and 8.3% patients were PSD. 67 patients were male. Mutations co-occurred with the factor 5 mutation or the factor 2 in 16 cases. Thrombotic locations were as follows: multiple veins ($n = 8$), cerebral veins/embolic stroke ($n = 29$), deep veins (DVT; $n = 67$), DVT & pulmonary embolism ($n = 36$). The rates of provoked TE were 46.5% [C], 71.8% [adolescents] and 76.3% in adults ($P < 0.001$). A positive family history was present in 55.5% [C], 53.8% in adolescents and 80.0% [A] ($P = 0.007$). After withdrawal of anticoagulation, the odds of recurrence adjusted for ATD, PCD and PSD, combined defects, age at onset, provoked TE and a positive family history was significantly elevated in adults compared to children [OR/95%CI: 8.07/1.7–38.8] with no difference within the childhood cohorts.

Conclusion: Clinical phenotypes of ATD, PCD and PSD in children and adults showed similarities as well as differences. Among the differences a higher rate of unprovoked TE was found in children, whereas adults presented with a higher rate of a positive family history and a higher rate of recurrence. The latter should be taken into account when conducting prospective studies in these high risk patient groups.

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PO507-WED

Acquired von willebrand disease in a patient with underlying beckwith-wiedemann syndrome

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Background: von Willebrand disease (vWD) is the most common inherited bleeding disorder; however acquired vWD is very rare. Cardiac diseases, oncological diseases, hypothyroidism, SLE and medications used are among the underlying causes of acquired vWD.

Aims: Herein, we report a patient with Beckwith Wiedemann Syndrome (BWS) who developed acquired vWD.

Methods: A 4-year old boy was referred related to prolonged bleeding subsequent surgery for intestinal obstruction.

Results: The diagnosis of BWS was established at newborn period and Wilms tumor was diagnosed at right kidney at 1-year of age. Partial nephrectomy without any bleeding complication was applied. Subsequent to chemotherapy, the patient had uneventful course upto admission for abdominal pain and vomiting 2 years after completion of treatments for Wilms tumour. The surgical exploration revealed intestinal obstruction and perforation related to adhesion band and resection-anastomosis was performed. Related to prolonged and severe bleeding during surgery, he was referred and the laboratory evaluations revealed: aPTT 31.2 sec, INR 1.2, vWF-Ag 11.6%, Ristocetin cofactor activity 46%, factor VIII 59%, in-vitro bleedingtime (PFA) prolonged. A diagnosis of acquired vWD was established. The evaluations for primary tumour relapse or other malignancies were unrevealing and the during the follow-up of 1 year the patient did not develop any other malignancy.

Conclusion: Acquired vWD usually has a mild course, but may cause severe bleeding after surgical procedures. The correction of the underlying disease commonly corrects the acquired vWD. In our patient acquired vWD was diagnosed during the remission of Wilms tumour for 2 years. Additionally, during the partial nephrectomy the patient had no bleeding complication. The acquired vWD may precede the

overt tumour development; however in our patient during the 1 year of follow-up, he developed no recurrence of Wilms tumour or other malignancies.

Disclosure of Interest: None declared.

PO508-WED

Novel homozygous mutation in MCFD2 and heterozygous small deletion in LMAN1 genes causing combined factor V and VIII deficiency

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Background: Combined factor V and VIII deficiency is a rare cause of hereditary coagulation disease.

Aims: Herein, we report a patient with combined factor V and VIII deficiency who was diagnosed subsequent to severe bleeding after tonsillectomy.

Methods: A four-year old girl was referred to hematology department related to prolonged and severe bleeding subsequent tonsillectomy. The parents were first degree cousins.

Results: The personal history revealed easy and spontaneous bruising throughout the life. Hemogram revealed: Hgb 10.9 g dL⁻¹, WBC: 5.2×10^9 /L, platelet: 216×10^9 /L. aPTT 75 ms, PT 19.9 ms, INR 1.8. Anti-cardiolipin, anti-phospholipid antibodies and lupus anticoagulant were negative. Fibrinogen was measured as 241 mg dL⁻¹. Factor V: 13% and factor VIII: 14%. The molecular studies of the patient revealed novel mutations in both *MCFD2* (homozygous c.[91–112dup];[91–112dup]) and *LMAN1* (heterozygous c.[539 + 11–539 + 12delGT] genes).

Conclusion: Combined factor V and VIII deficiency is rare and the molecular characterization of disease has rarely been reported. Further data on molecular characteristics of these patients will increase our knowledge about the presence of any genotype-phenotype correlations. Additionally, these patients may have severe bleeding during surgery if operated without any preventive measures. Among the possible perioperative management options are DDAVP, fresh frozen plasma, tranexamic acid and factor VIII concentrates.

Disclosure of Interest: None declared.

PO509-WED

Treatment of pediatric thrombosis with dalteparin sodium

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Background: There is only small amount information about using Dalteparin for treatment of pediatric thrombosis

Aims: Our aim was to evaluate safety, efficacy, and estimate doses of Dalteparin in children, treated because of thrombosis.

Methods: We observed 101 children suffering from thrombosis, who were treated with Dalteparin sodium. We analyzed case history 62 of them, 39 had no enough information for analysis. All of the patients received 2 times subcutaneous injections. The initial doses were corrected up to target value of anti-Xa-activity 0.5–1.0. Duration of treatment of venous thrombosis was from 6 weeks till 6 months, for arterial thrombosis – from 4 weeks till 3 months. The observation

period was from 1 year till 3 years. We provide the substitutive treatment for patients with activity of antithrombin III level less than 50% with FFP or antithrombin III concentrate.

Results: Age of the patient was: less 1 month old – 21; 1 month old – 6 month old – 9; > 6 month old – 17 years old – 32. Localizations of thrombosis were: vena cava inferior and superior – 4; intracranial veins – 9; portal vein – 5; renal veins – 2; other veins – 33; arterial thrombosis – 3; intracardiac – 7; ischemic stroke with thrombi in cerebral arteries – 2. The middle final dose of Dalteparin sodium in the newborns was $133 \pm 20 \mu\text{kg}^{-1}$; for other $120 \pm 17 \mu\text{kg}^{-1}$ with no valid differences between the groups. We analyzed results of the treatment in 56 patients (5 children didn't come back for catamnesic examination). The results were: full recanalization – in 31 patients (54.4%); partial recanalization – in 23 (40.3%); no recanalization – 3 (5.3%). No heparin-induced thrombocytopenia and serious bleedings observed in the patients. 15 patients had mild bleedings, which didn't need in correction of dose or stop the treatment with Dalteparin. It was possible to provide home treatment with Dalteparin for all of the patients.

Conclusion: Dalteparin is relatively safety and effective for treatment of children with thrombotic events.

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PO510-WED

Neonatal and adult platelets reveal a distinct protein expression profile?

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Background: It has been suggested that neonatal platelets are less reactive than platelets from adults. A reduced expression of specific platelet integrins or glycoproteins has been proposed to cause this phenomenon. Next to these inconclusive observations, decreased platelet functioning has not been directly associated with an increased risk for bleedings in healthy newborns. Together, this indicates that the reduced reactivity of neonatal platelet is poorly understood.

Aims: To increase insight in neonatal platelets, this study aims to obtain an overview of the differential protein expression between neonatal and adult platelets using a label-free quantitative mass spectrometry approach.

Methods: Platelets were isolated from cord blood of five neonates and from blood of six adults. Platelet proteins were processed by in-solution trypsin digestion and the resulting peptides were analyzed using a nano-LC Orbitrap Fusion mass spectrometer. The differential protein expression was assessed by comparison of chromatographic peptide ion intensities derived from the processed neonatal and adult proteins.

Results: High protein sequence coverage was obtained for the critical platelet integrins, glycoproteins, a-granule proteins, and for proteins that contribute to integrin activation. However, there was little variation in the level of these protein between the neonatal and adult platelets. The vitamin K-dependent protein S was among the down-regulated proteins in the neonatal platelet proteome. The upregulated proteins included thromboxane A2 synthase and enzymes involved in metabolic processes.

Conclusion: Although there is a differential protein expression between neonatal and adult platelets, no apparent difference is observed in the protein level of platelet integrins, glycoproteins, and a-granule proteins. We propose that a mechanism that is independent of the level of

expression of integrins and/or glycoproteins causes the reduced reactivity of neonatal platelets.

Disclosure of Interest: None declared.

PO511-WED

The influence of childhood immunizations on warfarin effect

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Background: Immunizations in children are a component of routine pediatric care. Complicating this is the significant numbers of children on warfarin therapy. Warfarin is known to be affected by a number of factors including diet, illness (immune response) medication, and error (missed doses). In addition there is concern about adverse events (drug-drug interactions and bleeding (hematomas at injections site) post immunization in children on therapeutic anticoagulation.

Aims: To determine how warfarin effect is influenced by immunization, and to determine the risk of bleeding post injection (local (IM) and systemic).

Methods: This retrospective cohort study included children followed by the KIDCLOT program, who were prescribed warfarin therapy with stable INRs within range for ≥ 1 month, and received an immunization. Warfarin was not interrupted pre /post immunization and parents were instructed to apply firm pressure to the immunization site for 5–10 min post injections. INRs pre and post immunizations (within 7 days) were collected. Patients were instructed to notify KIDCLOT of their immunization and any adverse events within 1 week.

Results: 14 consecutive patients received 33 immunizations of 8 types. There were 7 males. Underlying diagnosis was acquired ($n = 1$) and congenital heart disease. All INRs were within target range pre and post immunization. Mean INR pre/post immunizations were 2.3 SD 0.46 and 2.4 SD 0.46, respectively although a significant increase in INR ($P < 0.019$). There were no adverse bleeding events including hematomas.

Conclusion: Non-interruption of warfarin therapy appears to be safe when administering IM immunizations. The INR was not significantly affected by any immunization. Applying pressure post immunization may reduce the occurrence of hematomas. A registry confirming this safety may be useful following introduction of new anticoagulant agents.

Disclosure of Interest: None declared.

PO512-WED

Niacin has limited effectiveness in reduction of elevated lipoprotein(A) in children with or at risk of thrombosis or stroke

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Background: Elevated levels of lipoprotein(a) have been reported as a risk factor for stroke and arterial and venous thrombosis in adults and children. Niacin has been used to lower lipoprotein(a) in adults. Data is scarce in the pediatric population regarding the effectiveness of niacin treatment of elevated lipoprotein(a).

Aims: The aims of the study were to investigate the effectiveness of niacin on reducing lipoprotein(a) in children and to evaluate if treatment with niacin reduced the risk of recurrence of venous or arterial thrombosis or stroke.

Methods: A retrospective cohort of children, 0–18 years, was assembled if a lipoprotein(a) of $> 30 \text{ mg dL}^{-1}$ was observed after review of all lipoprotein(a) results obtained from January 1, 2005 to December 31, 2014. Statistical methods included Wilcoxon rank sum test, Chi Square test and logistic regression (SAS v9. 4, Cary, NC, USA).

Results: Of the 111 evaluable children, 26% (29/111) had isolated elevated lipoprotein(a) and 74% (82/111) had a thrombotic event: 33 had stroke, 6 had arterial thrombosis and 43 had venous thromboembolism. The lipoprotein(a) levels were obtained for personal or family history of thrombosis or cardiovascular disease. Of the 111 children, 75 had at least one other laboratory confirmed thrombophilic risk factor. Twenty-nine children were treated with niacin with a median starting dose of 100 mg BID (50–1000 mg daily-BID). The median maximal tolerated dose (MTD) was 100 mg BID (0–2000 mg) with a median time to MTD of 3 months (0–60 months). Twenty three children had available pre and post treatment levels. No significant difference in levels was seen after treatment with niacin. Effectiveness did not differ by dose, gender or race. Thrombosis or stroke recurred in 7 (6%) children, of which 1 had received treatment with niacin.

Conclusion: Elevation of lipoprotein(a) is commonly seen in children with thrombosis or at risk for thrombosis. Treatment with niacin was not effective in this cohort and warrants further investigation in a prospective study.

Disclosure of Interest: None declared.

PO513-WED

Hemostasis profile in calves receiving the carmat bioprosthetic artificial heart

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Background: The Carmat total artificial heart (TAH) is an implantable, electro-hydraulically driven, pulsatile flow device with four bioprosthetic valves. Its blood-pumping surfaces consist of processed bioprosthetic pericardial tissue and expanded polytetrafluorethylene (ePTFE), potentially allowing a reduction of anti-coagulation.

Aims: In preparation for clinical studies, we evaluated the TAH performance in an animal model and explored effect of TAH implantation on hemostasis parameters.

Methods: Twelve female Charolaise calves, 2–3 months of age and weighing 102–122 kg, were implanted with the TAH through a mid-sternotomy to ensure an adequate anatomic fit. 4/12 calves had a support duration of several days (4, 4, 8 and 10 days) that allowed us to explore hemostasis parameters. We explored prothrombin time (PT), coagulation factors (II, V, VII+X and fibrinogen) and platelets count. In these calves, we also performed multimeric analysis of von Willebrand factor (VWF) to detect a potential loss of high molecular weight (HMW) multimers, like previously described in some cardiac assistance device.

Results: Despite absence of anticoagulation in postoperative phase, no sign of coagulation activation was detected. Indeed, after an immediate post-surgery decrease of PT, platelets and coagulation factors, all

parameters went back to acceptable normal pre-surgery values. Evaluation of HMW multimers of willebrand factor did not show any modification after Carmat TAH implantation either after initiation or during days of support duration.

Conclusion: The Carmat bioprosthetic TAH implanted in calves up to 10 days provided adequate blood flow to tissues. The recuperation of coagulation parameters and platelets in post-operative phase without the use of anticoagulation validates the *in vivo* haemocompatibility of this TAH. Moreover, we did not observe any acquired von Willebrand syndrome after the Carmat TAH implantation.

Disclosure of Interest: None declared.

PO514-WED

Pediatric stroke occurrence in one children hospital in cordoba, argentina

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Background: Pediatric stroke (PS) is a rare event, frequently undiagnosed or misdiagnosed. Incidence of pediatric arterial ischemic stroke (AIS) is 2,4/100,000 children under 18 years. There are no data in Argentina. There is a male predominance; 25% of children have multiple risk factors associated. PS leads to significant morbidity and mortality, 25% will die, 25% will have a recurrence, and others will experience neurological deficits.

Aims: Description of 11 cases of PS.

Methods: Retrospective and descriptive study, involving children (range 1 month- 16 y.) who suffered AIS, assisted during 2009–2014 at the Hospital Infantil, Córdoba.

Results: Eleven children were included, of which 54% were male, with a median age of 9 years (range 0, 5 - 13 y.). Clinical findings: 54% experienced seizures, 54% presented focal neurological signs: hemiparesis (6), visual disturbance (1), speech disturbance (1), facial palsy (1), and 9% showed signs of reduced level of consciousness. Risk factors present in 72% of the cases: infection (3, varicella [1]), cardiac disorders (1), history of TIAs at time of diagnosis (1), trauma (2), radiotherapy (1), prothrombotic states (3) and dehydration (2). A third of children had multiple risk factors. Cerebrovascular imaging obtained in all cases: CT (11), MRI (7), MRA (2) and angiography (2). Lesion location: Frontal region found the most affected area (42%) and 63% suffered multiple infarcts. One child showed hemorrhagic component to the infarct. Thrombophilia tests performed in 72%: heterozygous for MTHFR (1), heterozygous for P20210 (1), low antithrombin III secondary to nephrotic syndrome (1). Morbidity and mortality: 4 children experienced impairment cognitive ability and social participation and 2 suffered from epilepsy. There was one recurrence, one patient died.

Conclusion: Early recognition of pediatric stroke, concerning signs and symptoms and significant risks factors, will improve the prospects to more adequate and rapid treatment, as well as care outcomes of childhood AIS.

Disclosure of Interest: None declared.

PO515-WED

Dose requirements of acenocoumarol in paediatric patients – initiation of THERAPY

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Background: Results from dose response studies to acenocoumarol (AC) in childhood are far from being sufficient.

Aims: To establish effect of age on dose response to AC and on achievement optimal coagulation control in children during initiation period of therapy.

Methods: Retrospective analysis was done on 188 patients from Pediatric cardiac surgery during period from 2005 to 2013, 61 girls and 127 boys, average overall age 8 ± 5.4 years. AC was administered as a primary prevention of thromboembolic adverse effects in 96% of the patients: 76 (40%) with valve prosthesis, 65 (35%) with Fontan procedure, 33 (18%) with conduit for biventricular correction, and 6 (3%) with other procedures. INR range between 2.5 and 3.5 was set in patients with mechanic valves in mitral position (7%), whereas INR range between 2.0 and 3.0 (93%) was set for all other indications. Prothrombin time was measured on STA Compact, using STA Neoplastin Plus with ISI of 1.2.

Results: Highest loading dose of AC of $0.138 \pm 0.08 \text{ mg kg}^{-1}$ ($n = 20$) was required by the children of age below 2 years in comparison with the other three age groups ($P < 0.001$). Children aged > 2 £5 years ($0.114 \pm 0.027 \text{ mg kg}^{-1}$, $n = 59$) and > 5 £13 years ($0.093 \pm 0.029 \text{ mg kg}^{-1}$, $n = 63$) continue to demonstrate a trend to higher loading dose in comparison with children at age > 13 £18 years who required the lowest average loading dose of $0.069 \pm 0.02 \text{ mg kg}^{-1}$ ($n = 45$). About 60% of all INR tests were in therapeutic range on the fifth day from the therapy initiation in all study groups. Supporting dose for the period between 7th and 30th day after hospitalization was Gr1 0.126 ± 0.103 , Gr2 0.054 ± 0.025 , Gr3 0.051 ± 0.020 , Gr4 $0.037 \pm 0.016 \text{ mg kg day}^{-1}$.

Conclusion: On the basis of linear regression models, describing role of age, weight, basal INR, loading dose, as well as interactions between them, the following formula was developed: Loading dose (mg kg^{-1}) = $(0.692 - 0.007 * \text{age} - 1.035 * \ln(\text{basal INR})) * 0.241$.

Disclosure of Interest: None declared.

PO516-WED

Direct thrombin inhibitor for anticoagulation: role of argatroban in pediatric post liver transplant patients with hepatic artery thrombosis

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Background: Anticoagulation strategies are challenging in children undergoing liver transplantation due to an increased risk of bleeding and variable responses to standard heparin or low-molecular-weight heparins. Use of standard unfractionated heparin and enoxaparin therapy has been reported in the setting of post-LT thromboembolic events including Hepatic Artery Thrombosis (HAT). Management remains challenging due to bleeding concerns.

Aims: Herein, we make the first report of argatroban in 3 pediatric LT patients for the management of post-LT thrombosis without bleeding complications after failure of standard anticoagulation management or suspicion of HIT.

Methods: Case 1: 7 mo with biliary atresia/Factor V Leiden mutation, post 2nd LT developed poor hepatic artery flow within hours of surgery on prophylactic heparin. He responded to surgical repair and therapeutic heparin. Week later, with HIT concern and new HAT finding, Argatroban/aspirin were initiated with resolution of HAT. Case 2: 10 mo undergoing LT for residual Hepatoblastoma developed HAT. Argatroban restored HA flow with 24 h. Case 3: 12 mo with biliary atresia/Protein C deficiency on prophylactic enoxaparin, developed HAT. Surgical revascularization and thrombectomy was performed. Intraoperative vasospasm and re-clotting prompted argatroban/aspi-

rin therapy with successful resolution of HAT. Results: The 3 patients were discharged with satisfactory liver function and normal hepatic arterial flow, now 60 to 388 days in follow up. Reduced dose Argatroban can be used in setting of HAT in pediatric LT patient and tolerated without major bleeding complications. In our experience, argatroban has proved efficacious and relatively simple to use.

Conclusion: Use of reduced-dose Argatroban in pediatric liver transplant patients who have failed standard management or where there are concerns about possible HIT can be done without excessive bleeding in our limited series. This is an area where further studies are needed.

Disclosure of Interest: None declared.

Rare bleeding disorders II

PO517-WED

Characterization of six alterations in ITGA2B and ITGB3 associated with glanzmann's thrombasthenia

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Background: Glanzmann's thrombasthenia (GT) is an inherited bleeding disorder caused by defects in the α IIb β 3 receptor which mediates platelet aggregation at sites of injury. The α - and β -subunits of α IIb β 3 are encoded by *ITGA2B* and *ITGB3* respectively. We have identified four *ITGA2B* and two *ITGB3* missense alterations associated with GT in a UK patient cohort.

Aims: To confirm the pathogenicity of amino acid substitutions in the α IIb (D396N, L492P, I596T and N670K) and β 3 (C547W and I665T) subunits in GT.

Methods: The effects of candidate mutations were predicted using online tools and structural modelling. Expression of recombinant α IIb β 3 receptors, and their ability to bind PAC1 and fibrinogen after activation, was examined by flow cytometry of Chinese Hamster Ovary cells following co-transfection with wild type (WT) or variant *ITGA2B* and *ITGB3* expression vectors.

Results: All six candidate mutations were predicted to be deleterious. Compared to cells expressing WT α IIb β 3, there was an almost complete absence of surface α IIb β 3 in cells expressing the D396N and I596T α IIb variants ($P < 0.0001$), while the L492P α IIb and C547W β 3 variants showed 60% ($P < 0.0001$) and 40% ($P < 0.001$) reductions in surface expression respectively. The L492P α IIb variant failed to undergo activation ($P < 0.0001$), while the C547W β 3 variant showed spontaneous binding to PAC1 and fibrinogen ($P < 0.0001$). The N670K α IIb and I665T β 3 variants were expressed at similar levels to WT, but showed reduced ability to bind PAC1 ($P < 0.0001$ & $P < 0.01$) and fibrinogen ($P < 0.01$ & $P < 0.05$).

Conclusion: Our findings show that the D396N, L492P and I596T substitutions are likely to cause reduced expression of α IIb β 3 on the platelet surface, while the N670K and I665T substitutions result in α IIb β 3 that is expressed normally, but has impaired activation. The C547W substitution results in reduced expression of an α IIb β 3 receptor that is locked in a high-affinity conformation which allows spontaneous ligand binding. Our data confirm the pathogenicity of these variants in GT.

Disclosure of Interest: None declared.

PO518-WED

Two families from argentina with FX deficiency due to two different mutations (P.CYS241GLY and P.GLN138ARG)

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Background: Coagulation factor X (FX) deficiency is a rare autosomal recessive disease and is one of the most severe among the rare bleeding disorders. Very few cases of congenital FX deficiency have been reported from South America.

Aims: We have detected two unrelated families from the Cordoba area in the centre of Argentina whose members presented two new mutations.

Methods: Family A (GM): The proposita is a 41 year old female who has had all her life a moderate bleeding tendency. Parents were not consanguineous and their background was Spanish –Argentinean. Family B (FD): The proposita was a 51 year old female who had a mild bleeding tendency. Parents were not consanguineous and had an Italian-Argentinian background. Prothrombin time (PT), Russell's viper venom (RVV) and aPTT were assayed using standard techniques. FX activity was determined using a one-stage clotting assay based upon PT, and FX chromogenic level was measured using commercial kit (Hyphen France). Coding regions and exon/intron boundaries of FX were amplified in DNA samples by PCR and the products obtained were sequenced.

Results: The proposita GM showed a prolongation of aPTT, PT and RVV. There was no circulating anticoagulant. The FX clotting activity level was 3% of normal and chromogenic of 0.04 IU mL⁻¹. Genetic analysis showed a p.Cys241Gly mutation in exon 6. Other family members presented the same mutation. The patient FD the aPTT, PT, and RVV were prolonged and the FX clotting activity was 2% of normal and chromogenic 0.03 IU mL⁻¹. Again there was no circulating anticoagulant. Genetic analysis revealed the presence of a p.Gln138Arg mutation in exon 5. Other family members showed the same mutation.

Conclusion: These are the first FX mutations reported in Argentina. Extensive family studies are underway in order to define the diffusion of these two defects in the local population. Since the two mutations have never been described in Spain or Italy or in any other part of the world, it is plausible that the founder was Argentinean.

Disclosure of Interest: None declared.

PO519-WED

Platelet type - von willebrand disease (PT-VWD): a diagnostic and therapeutic dilemma in children

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Background: PT-VWD (platelet type - Von Willebrand disease) is indistinguishable from VWD-Type 2B clinically. While laboratory data can providesome insight, genetic analysis revealing mutations in the *GP1BA* gene remains the confirmatory test. Diagnosis of this disease in childhood is often based on family history of the disease or personal bleeding history. The PT-VWD registry (www.pt-vwd.org) reports 52 cases of which only two patients were diagnosed in childhood without a family history.

Aims: To report two pediatric PT-VWD cases diagnosed at our institution.

Methods: Case 1 An eight -month old boy diagnosed with neonatal allo-immune thrombocytopenia (NAIT) at birth refractory to platelet transfusions and intravenous IgG presented with a cephalohematoma after a fall. Investigations revealed thrombocytopenia, VWF:RCo/Ag ratio of 0.38, enhanced low dose ristocetin –induced platelet aggregation (RIPA) and clinical response to VWF containing concentrate consistent with VWD-2B. Both parents were clinically asymptomatic with normal VWD panel and RIPA workup. Case 2 A newborn girl with history of VWD-2B in father presented with thrombocytopenia and normal VWF:RCo/Ag ratio and multimers at birth. Her platelet counts normalized in infancy; however, she had occasional palpable bruises. A repeat VWD panel at 1 year revealed a ratio of 0.2 and enhanced RIPA suggestive of VWD-2B.

Results: The genetic testing in both cases was negative for *VWF* gene-exon 28 mutation and revealed p.Met255Val of *GP1BA* gene confirming the diagnosis of PT-VWD. This is the first report of PT-VWD misdiagnosed as NAIT which highlights the importance of accurate diagnosis to prevent alloimmunization to platelets on exposure during early childhood.

Conclusion: Diagnosis of PT-VWD in infancy and early childhood can be challenging particularly in the absence of family history or bleeding symptoms. This emphasizes a high index of suspicion for PT-VWD in cases of unresolved NAIT in order to formulate optimal treatment strategies.

Disclosure of Interest: None declared.

PO520-WED

Rare bleeding disorders: 17 years of diagnosis in the hemostasis laboratory of the hematology department of the university hospital of beni messous, algiers, algeria

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Background: Algeria is an emerging country of North Africa where the frequency of rare bleeding disorders (RBDs) increases because consanguineous marriages are still customary.

Aims: The aim of our study is to appreciate the frequency of RBDs, the severity of the factor deficiencies & the clinical manifestations in the patients we diagnosed.

Methods: We tested patients with bleeding disorders &/or abnormal hemostasis tests, we realized PT/APTT & the specific dosages of clotting factors. Patients were tested at least twice on different blood samples, we eliminated acquired hemostasis anomalies. Our laboratory participates to an international external quality assessment program. Clinical symptoms & histories were registered & family trees were realized.

Results: In 17 years of activity, 322 patients (range age from new born to 86 years old) were diagnosed: 19 FI, 06 FII, 68 FV, 163 FVII, 34 FX, 1 FX associated with von Willebrand disease type 1, 08 FXI, 12 FXII, 01 FXIII, 02 FV/FVII, 03 FV/FVIII, 04 FV/FX, 01 FVII/FX, from mild to severe. A positive bleeding history was found in 71 patients (22%) & more frequently in homozygous factor deficiencies. The most common sites of bleeding were skin & mucous membranes.

Conclusion: RBDs are surely under detected in Algeria because of variable nature of relatively mild symptoms & the lack of adequate hemostasis laboratory means. Education & genetic counseling are needed to prevent & limit the spread of such occurrence of RBDs, moreover because factor concentrates are expensive & not available for some deficiency states.

Disclosure of Interest: None declared.

PO521-WED

Factor XIII deficiency: preliminary results of the pro-rbdt project

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Background: The PRO-RBDD is a 3-year prospective data collection.

Aims: It aims at understanding the association of FXIII activity and clinical severity in patients with FXIII deficiency. Benefits and complications of on-demand and prophylactic treatment were also evaluated.

Methods: Data were collected at baseline (historical) and every 6 months (follow-up). 56 patients (31 females, 25 males), of whom 8 children (≤ 12 years) were recorded

Results: Regression analysis confirmed a strong association between FXIII levels and age of diagnosis (Pearson test $P < 0.05$) or clinical severity (classified according to Peyvandi et al, JTH 2012) ($\beta = -12.95$, $P < 0.05$). Grade III bleeding were observed in 87% (20/23) and 67% (8/12) of patients with severe (undetectable activity) and moderate (5–30%) deficiency. More than 1/3 of patients with mild ($> 30\%$) deficiency were asymptomatic and none did have grade III bleeding. Mucocutaneous bleedings, hematoma and haemarthrosis were present in almost 1/3 of patients, regardless their FXIII coagulant levels. More severe symptoms such as CNS, GI and umbilical cord bleeding were observed only in severe and moderate deficiency (13, 4 and 20%). Prophylaxis regimen with plasma-derived FXIII concentrates was used in 2/3 of severe patients and 1/3 of moderates. On 27 women, only 2 had menorrhagia; on 50 pregnancies, 25 ended to a spontaneous abortion and 5 showed bleeding complication. Follow-up data (up to 700 days) on 31 patients on “on-demand” therapy and on 18 on prophylaxis (9–57 U/Kg/month FXIII concentrate) showed an incidence bleeding rate of 1.8 patient-year⁻¹ (95%CI 1.3–2.3) and 0.15 (95%CI 0.02–0.36), respectively. The risk of bleeding requiring replacement therapy is significantly higher in patient on “on-demand” (log rank test = 0.05). No thrombotic events were observed.

Conclusion: The PRO-RBDD clearly confirmed the beneficial role of FXIII prophylaxis with no bleeding and no thrombotic events, making this treatment strongly recommended

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PO522-WED

Effects of factor XIII deficiency on thromboelastography. basal thrombelastograph and lysis by addition of streptokinase are more affected than solubility tests

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Background: Severe factor XIII (FXIII) deficiency is characterized by soft tissue hematomas, intracranial and delayed spontaneous bleeding. Alterations of Thromboelastographic (TEG) parameters in these patients have been reported.

Aims: To show results of TEG in whole blood, TEG Lysis (Lys) by subthreshold concentrations of streptokinase (SK), and clot solubility studies in samples of a patient with severe FXIII deficiency, and to compare TEG and solubility tests of different mixtures of FXIII deficient and normal plasmas.

Methods: We studied a 1 year old girl derived to our center because of bleeding from umbilical cord and important hematomas in soft tissue. Clotting tests, vWF:Ag and activity, XIIIa subunit (immunoturbidimetric assay) were performed in ACL TOP coagulometer (Instrumentation Laboratory). Solubility tests were performed with Ca²⁺ + 5M urea and thrombin-2% acetic acid. Basal and post recombinant FXIII concentrate infusion samples were studied. TEG was performed with CaCl₂ or CaCl₂ + SK (3.2 U mL⁻¹) in a Thromboelastograph D Hellige.

Results: PT, APTT, TT, fibrinogen, VIIIc, vWF and platelet aggregation were normal. Antigenic XIIIa subunit was $< 2\%$. TEG presented a normal R (8 min), prolonged k (14 and 11 min), low MA (39 and 52 mm) and Ly60 slightly increased (23 and 30%) in basal and post factor XIII concentrate infusion (XIIIa = 37%), respectively. Basal clot solubility was abnormal 50 and 45 min with Ca-Urea and thrombin-acetic acid, respectively, but normal (> 16 h) after FXIII infusion. Analysis of FXIII deficient and normal plasma mixtures ($< 2 - 102\%$ of XIIIa), showed that Ca-urea solubility was abnormal at XIIIa $< 9\%$, thrombin-acetic acid at XIIIa $< 18\%$, but TEG MA, shear force and Lys60 with and without SK at XIIIa $< 40\%$.

Conclusion: TEG parameters and Lys60 by SK are more sensitive to low levels of FXIII than solubility tests, and could be one possible explanation for the bleeding manifestations reported in many patients with XIII levels between 5–40%.

Disclosure of Interest: None Declared.

PO523-WED

High molecular weight kininogen deficiency: a report of eleven venezuelan cases

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Background: High-molecular-weight kininogen deficiency (HMWKD) is a very rare hereditary disorder that is inherited by an autosomal recessive manner. The disorder is not associated with abnormal bleeding; rather, some reports suggest that HMWK deficiency contributes to thrombosis. Individuals with this condition usually exhibit an isolated and very prolonged activated partial thromboplastin time (APTT); diagnosis could be complicated by the presence of other coagulation defects.

Aims: Clinical and laboratory analysis of our HMWKD patients.

Methods: We reviewed the patients' records attended in our center since 1998 to 2014. We identified 11 HMWKD cases. Screening Tests: PT, APTT, TT (Stago reagents, CK Prest for APTT), platelets and FXIII performed according classical techniques. Confirmatory tests: FXII, FXI, FVIII, FIX by one stage method (deficient plasma IL); HMWK (Technoclone), FvWAg (Elisa), Lupus anticoagulant (IL), and the APTT with 10 min of activation for the Prekallikrein testing.

Results: We reviewed the patients' records attended in our center since 1998 to 2014. We identified 11 HMWKD cases. Screening Tests: PT, APTT, TT (Stago reagents, CK Prest for APTT), platelets and FXIII performed according classical techniques. Confirmatory tests: FXII, FXI, FVIII, FIX by one stage method (deficient plasma IL); HMWK (Technoclone), FvWAg (Elisa), Lupus anticoagulant (IL), and the APTT with 10 min of activation for the Prekallikrein testing.

Conclusion: Diagnose of the HMWK deficiency is based on the observation of a very prolonged APTT in generally asymptomatic patient; the coexistence with other haemostatic defects and the presence of bleeding symptoms may make complicated its diagnosis; Discrepancies between clinics and tests results led us to identify multiple defects. Identify molecular defect will allow confirmation of this rare deficiency

Disclosure of Interest: None declared.

PO524-WED

Diagnosis of FXIII deficiency: data from multicentre studies amongst UK NEQAS and PRO-RBDD project laboratories

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Background: FXIII deficiency is a rare bleeding disorder, and specific FXIII assays are recommended to detect this deficiency. Multicentre UK NEQAS exercises have demonstrated a move from clot solubility screening tests to FXIII assays, improving diagnostic performance.

Aims: In this study, we investigated the performance and diagnostic accuracy of FXIII investigations in centres enrolled in the PRO-RBDD project (prospective data collection on patients with fibrinogen and Factor XIII deficiencies), in comparison to UK NEQAS centres.

Methods: Samples from a FXIII deficient subject and a normal donor were sent to 141 UK NEQAS and 29 PRO-RBDD centres, who were asked to perform their routine investigation for FXIII deficiency, and interpret their results. Median, coefficient of variation and range were determined for each assay method.

Results: Results were returned from 98 UK NEQAS and 21 PRO-RBDD centres. 39 UK NEQAS and 11 PRO-RBDD centres reported clot solubility screen results – diagnostic errors were made by one NEQAS centre (false negative for the FXIII deficient sample) and one PRO-RBDD centre (false positive for the normal sample). 66 UK NEQAS centres and 15 PRO-RBDD centres reported FXIII assay results. Medians (range, CV) were as follows: 4.6 μdL^{-1} (0.0–107.0 μdL^{-1} , 195%) and 3.9 μdL^{-1} (0.0–11.0 μdL^{-1} , 69.4%) for the FXIII deficient sample and 108 μdL^{-1} (15.6–148.0 μdL^{-1} , 15%) and 103 μdL^{-1} (47.0–127.0 μdL^{-1} , 20.4%) for the normal sample respectively. Diagnostic errors were made by 2 UK NEQAS centres.

Conclusion: Approximately 70% of both UK NEQAS and PRO-RBDD centres now employ FXIII assays, in keeping with international recommendations. However, solubility tests continue to be used. Our data show that this can be successful in some centres, depending on the sensitivity of the method in use. Diagnostic errors are made by centres using both solubility screens and FXIII assays and laboratories should ensure good quality assurance procedures to improve diagnostic accuracy.

Disclosure of Interest: None declared.

PO525-WED

The clinical findings of children with factor x deficiency at a single center in the middle east of Turkey

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Background: Factor X (FX) deficiency is a rare, autosomal recessive bleeding disorder representing 8–10% of all rare bleeding diseases. Mucocutaneous bleeding symptoms, hemarthrosis, intracranial hemorrhage, and gastrointestinal bleeding may occur in patients causing a severe bleeding phenotype.

Aims: To present the clinical features, bleeding phenotype and treatment outcome of patients with FX deficiency followed-up at a single center in the Middle East of Turkey. Our center is a government hospital and has 124 factor deficiency patients registered.

Methods: We retrospectively analyzed files of 7 patients for bleeding symptoms, bleeding frequency and response to treatment.

Results: Six children had severe (< 10%) and one had moderate (10–40%) disease. In 3 patients symptoms started during neonatal period; one had muscle hematoma after vaccination, one had umbilical bleeding and one had spontaneous intra-abdominal bleeding. In others complaints started between 4 months–8 years of age (median: 1.6 years). The majority of symptoms were mucocutaneous bleeding. One patient (FX: 1.7%) had recurrent hemarthrosis, one (FX: 1%) had muscle hematoma and one (FX: 1.8%) had gastrointestinal bleeding. None of the patients had intracranial bleeding. Patients were treated with fresh frozen plasma (FFP) before 2013 and with prothrombin complex concentrate (PCC) after 2013. No patient developed thrombosis due to PCC. Three patients were put on prophylactic treatment with PCC due to excessive mucocutaneous bleeding, recurrent hemarthrosis and gastrointestinal bleeding. Prophylactic treatment decreased the frequency of bleeding in all patients.

Conclusion: Factor X deficiency is a rare bleeding disorder but prevalence may increase in populations where consanguinity is common.

Disclosure of Interest: None declared.

PO526-WED

Lupus anticoagulant - hypoprothrombinemia syndrome in patient - a case report

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Background: Lupus anticoagulant-hypoprothrombinemia syndrome (LA-HPS) is a rare acquired bleeding disorder caused by prothrombin antibodies. Bleedings vary in severity from mild to life-threatening. Laboratory results show prolonged activated partial thromboplastin and prothrombin time in combination with lupus anticoagulant.

Aims: LA-HPS - a case report and review of the literature.

Methods: male patient aged 35 with joint and muscle pain, exercise muscle haemorrhages for 10 years, periodic knee oedemas, bilateral spondylolysis, haustum of L5/S1 vertebra, antibody HCV present, thyroid adenoma, after one episode of an inflammatory of left bursa olecranon, chronic nicotine and marijuana burning for 10 years, he was admitted in good condition for hemorrhagic diathesis of upper and lower limbs and the inflammatory exudate of left bursa olecranon, no lymphadenopathy and hepatosplenomegaly were observed. The laboratory tests showed: WBC-3.11G/l, elevated gammaglobulins concentration (22.0 g L⁻¹), IgG antibodies and C3d complement on erythrocytes and heat autoantibodies were detected. The prothrombin time-25.76 s, INR-2.35, prothrombin index-41.15%, thrombin time-21.24 s, APTT> 160 s, factor II-20.10%, factor V activity-97.10%, factor VII activity-80.50%, factor X activity-75.58%, factor VIII activity-147.6%, factor XII activity-77.4%, antigen of factor von Willebrand-187.95%, factor von Willebrand's activity-212%, LA present (LA1 / LA2 -4.23), PFA 100CT:EPI-78s were measured using Siemens reagents. ROTEM Delta in-TEM and ex-TEM thromboelastometry showed essentially prolonged coagulation time and thrombogenesis time, normal maximal coherence of clot.

Results: No active bleeding diathesis was observed but nevertheless prolongation of the prothrombin, thrombin and activated partial thromboplastin time, occurred as well as decreased activity of factor II and presentation of LA.

Conclusion: Periodic active bleedings in muscles appeared after gym in fitness club and probably after marijuana burning.

Disclosure of Interest: None declared.

PO527-WED

Inherited FXI deficiency is not a rare disease in the northern region of Turkey

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Background: Inherited FXI deficiency is a bleeding disorder characterized by a decrease in the activity of FXI in plasma. It is inherited as an autosomal incompletely recessive trait. Therefore, both heterozygotes and homozygotes are at risk of excessive bleeding. Its prevalence in the northern Black Sea region of Turkey is unknown.

Aims: The aims of this study were to assess the prevalence of FXI deficiency in the northern region Turkey in children and adults with coagulopathy.

Methods: This study used retrospective data collected from hematology laboratories and electronic medical records. The data included 625 results of FXI assays that were performed between January 2005 and

January 2015 at the University Hospital. This hospital is a tertiary referral centre of the central part of the Black Sea region of Turkey, which has a population of approximately five million people. FXI activity (FXI:C) was performed by a one-stage method based on a modified partial thromboplastin time.

Results: During the total of 10 years period, 625 tests for FXI activity (FXI:C) were performed in our hematology laboratory. After 60 repeat tests assays were excluded, the data of the remaining 565 patients were evaluated. Inherited FXI deficiency was detected in 18.7% (106/565) of suspected patients. The median diagnoses age of the patients with FXI deficiency was 8.0 years (range, 2 days - 79 years). FXI activity levels were below 20% (severe deficiency) in 20 cases (19%), between 20 and 70% (mild deficiency) in 80 cases (81%). In severe deficiency group, there were 11 males and 9 females. Median FXI level was 6.5% (range, 4.9–18%).

Conclusion: In the northern region of Turkey, patients with inherited FXI deficiency are not as rare as expected. High ratio of consanguineous marriages in region may contribute this prevalence. Prospective population screening, that includes DNA analysis, is recommended to determine the prevalence of inherited FXI deficiency in this region and all Turkey.

Disclosure of Interest: None declared.

PO528-WED

Characterization of patients with a mild to moderate bleeding phenotype: results of the vienna bleeding biobank (VIBB)

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Background: Mild bleeding disorders (MBDs) find expression in various clinical phenotypes. Only an exact diagnosis allows targeted treatment.

Aims: The establishment of a biobank of clinically thoroughly characterized patients in order to identify underlying causes for MBDs.

Methods: To quantify the severity of the bleeding, a standardized bleeding score (BS) was used (Rodeghiero et al., JTH, 2005). Routine coagulation tests, von Willebrand factor antigen (vWF:Ag) and activity (vWF:RCo), single clotting factors, and platelet function, assessed by platelet function analyzer (PFA-100), aggregometry, and glycoprotein expression, were performed.

Results: From November 2009 to October 2014, 338 patients (82% female) with a median age [Interquartile range (IQR)] of 43 [32–56] years were included. The family history for MBDs was positive in 35% of patients. The median BS [IQR] was 5 [3–7] in the overall patient population. Definite von Willebrand disease (vWD, vWF:RCo < 30%) was diagnosed in 7 patients (2%), and suspected in 25 patients (7%, vWF:RCo 30–50%). A not further characterized definite platelet function disorder was diagnosed in 25 patients (7%) and was considered possible in further 27 patients (8%). FVIII deficiency with levels of < 50% was diagnosed in 11 patients (3%, < 20% in 2 of them, 2 females) and 1 patient each suffered from factor XIII deficiency (Factor XIII: 35%) and dysfibrinogenemia (Fibrinogen Clauss: 97 mg dL⁻¹). A large part of the patient population (241 patients, 71%, 82% female) showed normal test results or results not clearly associated with a bleeding disorder. The median BS was 5 (IQR 3–7)

in patients with or without a definite or assumed diagnosis of a bleeding disorder.

Conclusion: A definite or possible diagnosis explaining the bleeding tendency was made in 29% of patients with MBDs. The severity of bleeding, however, was comparable between patients with a definite or possible diagnosis and those without. Further mechanisms responsible for MBDs still have to be identified.

Disclosure of Interest: None declared.

PO529-WED

Clinicohematological profile of rare inherited coagulation disorders

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Background: The rare coagulation disorders are inherited abnormalities of hemostasis that may present significant difficulties in diagnosis and management. The overall frequency of these disorders in the general population is low and the clinical manifestations vary according to the type and magnitude of the deficient factor.

Aims: To analyze the clinicohematological profile of the rare inherited coagulation factor disorders diagnosed and followed up in a tertiary care hematology centre of North India

Methods: This was a retrospective study conducted in Department of Hematology, All India Institute of Medical Sciences, New Delhi, from 2012 to 2014. The cases were diagnosed based on the following tests, which include platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), relevant factors assays and clot solubility test in 5 M urea. Factor V, VII and X assays were PT based and factor XI assay was aPTT based.

Results: The cases include ten males and eight females. The median age of patient was 11 yrs (range; 6 months -45 years). The age at presentation ranged from eight days of age to 24 years (median - 04 years). The commonest manifestation include skin bleeds (72%) followed by soft tissue bleeding (55%). Commonest among the rare coagulation deficiency disorder include factor X (44%), followed by factor XIII (22%), factor VII (11%), Afibrinogenemia (11%), factor VII (5.5%) and dysfibrinogenemia (5.5%).

Conclusion: Many of these rare coagulation disorders present at very late age with oldest patient being 45 years of age. Also most of them present with cutaneous bleeding, mimicking platelet type of bleeding. Factor X deficiency is the most common among these rare coagulation disorders in our population. A high index of suspicion and an algorithm based diagnostic approach would aid in diagnosis of these rare coagulation disorders.

Disclosure of Interest: None declared.

PO530-WED

Clinical presentation and management of rare factor deficiencies: case study

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Background: Deficiencies of coagulation factors (other than factor VIII and factor IX) that cause a bleeding disorder are generally rare, with prevalences in the general population varying between 1: 500 000 and 1: 2 000 000. Owing to the relative rarity of these deficiencies, actual management of bleeding episodes are not as well established as haemophilia A and B.

Aims: In this study, we aim to report the clinical findings and treatment modalities of 27 patients with rare coagulation factor deficiencies at Çukurova University, Adana, southern part of Turkey.

Methods: We retrospectively reviewed medical records of 27 patients with rare coagulation factor deficiencies including FV, FVII, FX, Prothrombin deficiencies.

Results: 27 patients with rare coagulation factor deficiencies, age ranging from 3 months to eighteen years were included in the study. Three patients had prothrombin deficiency, three patients had FV deficiency, thirteen patients had FVII deficiency, and eight patients had FX deficiency. Mucocutaneous bleeding was the most common clinical presentation (65%). Intracranial haemorrhage (ICH) was seen in four patients in FX and two patients in FVII deficiencies. Of these, intracranial hematoma was evacuated in three patients. Other surgical operations including tooth extraction, circumcision, cataract extraction, tonsillectomy, osteotomy were performed in eight patients. The diagnosis was established in three patients after intracranial hemorrhage who were referred to our center with ICH. Prothrombin complex concentrates, recombinant factor VIIa (rFVIIa), fresh frozen plasma were used for bleeding control and surgery in these patients.

Conclusion: Laboratory and clinical findings of rare coagulation factor deficiencies are limited due to its low prevalence. Increased evidence-based guidelines for the diagnosis and management of this patient population is required. In this study, we reported the cases and have encountered in management of rare factor deficiencies.

Disclosure of Interest: None declared.

PO531-WED

Spectrum of rare bleeding disorders

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Background: Rare bleeding disorders include factor II, fibrinogen deficiency, factor XIII deficiency, factor V deficiency and factor X deficiency. It represents 5% of the patients with inherited bleeding disorders. Haemophilia A and B are the bleeding disorders commonly encountered in clinical practice. Von Willebrand disease although common, needs wide battery of specialised tests to diagnose all the subtypes and many of them can go undiagnosed if not investigated thoroughly. As a consequence of their rarity, clinical data regarding the characteristics of bleeding symptoms and their management remain uncertain.

Aims: To assess the prevalence of rare bleeding disorders and study the clinical features and analyse results of coagulation

Methods: The clinical and laboratory findings of cases diagnosed as rare bleeding disorders were reviewed and analysed. The tests performed were fibrinogen assay, factor VII, V, X assay and Clot solubility test for factor XIII.

Results: The clinical manifestation are heterogeneous. H/O consanguinity is commonly present. An analysis of all the bleeding disorders over a 5 year period showed 19 cases of factor XIII deficiency (1.8%), 23 cases of fibrinogen deficiency (2%), 9 cases of factor VII deficiency (0.9%), two cases of factor X deficiency, two cases of factor V deficiency, one case of factor XII deficiency. One case of combined factor V and factor VIII deficiency was diagnosed. One case each of Prekallikrein and HMW Kininogen were diagnosed based on APTT with extended incubation. 15 cases of Glanzmann's disease were diagnosed by flow cytometric confirmation of CD 41 negative platelets (1.6%).

Conclusion: Rare bleeding disorders can be suspected by simple step-wise approach to coagulation testing. Correct recognition of these deficiencies will help in initiating appropriate treatment

Disclosure of Interest: None declared.

PO532-WED

Congenital FXI deficiency: a report of nine families from germany and lithuania

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Background: Factor XI deficiency represents a rare coagulation disorder associated with mild bleeding tendency requiring no prophylactic treatment even in severe cases. Due to rareness of this disorder still too little is known about the perioperative or posttraumatic treatment strategy.

Aims: Genotyping, presentation of laboratory and clinical data of 16 patients with congenital FXI deficiency from Germany ($n = 14$) and Lithuania ($n = 2$).

Methods: Among standard coagulation tests a direct sequencing of *F11* gene and in one case *F8* gene has been performed.

Results: In total data of 16 individuals has been evaluated comprising one patient with severe deficiency, one patient with moderate deficiency and 14 patients with mild FXI deficiency (FXI 7 to 75% of normal). In total 11 unique (different) mutations have been identified comprising six missense mutations, four nonsense mutations and one small deletion. Interestingly, one asymptomatic patient from Lithuania besides mild FXI deficiency also showed congenital, mild Haemophilia A. In addition the other patient from Germany was known to have mild thrombocytopenia, unclassified platelet dysfunction due to impaired platelet aggregation and pathologic PFA test. Perioperative management was arranged by substitution of fresh frozen plasma and antifibrinolytic therapy. Because of allergic reaction against plasma products and platelet dysfunction two patients have been treated with recombinant FVIIa showing no bleeding or thrombotic complications.

Conclusion: This report demonstrates high genetic variability and phenotypic presentation of patients with FXI deficiency. To our knowledge this is the first report showing a combination of FXI and FVIII deficiency in the same individual.

Disclosure of Interest: None declared.

PO533-WED

Two female cases of A1- antitrypsin Pittsburgh

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Background: The $\alpha 1$ -Pittsburgh ($\alpha 1$ -AT) is an extremely rare bleeding disorder due to a Met358Arg mutation in $\alpha 1$ -antitrypsin gene, leading to $\alpha 1$ -AT become a potent inhibitor of thrombin rather than an inhibition of elastase. Up to date, only 4 patients has been reported.

Aims: To investigate the clinical characters of Pittsburgh mutation in two female patients and to detect $\alpha 1$ -AT gene mutation.

Methods: All plasma clotting assays were performed using commercial reagents from Diagnostica Stago. AT-III and protein C activities were measured by the synthetic chromogenic substrate method. Protein S activity was evaluated by a clotting assay. Platelet aggregation was evaluated by aggregometry. The Serum protein electrophoresis was performed on Sebia HYDRASYS by Agarose gel. The active region of $\alpha 1$ -AT was amplified by polymerase chain reaction (PCR) and then sequenced and blasted with NCBI gene bank.

Results: The proband was a 34-year-old female presented with uncontrolled bleeding after a cesarean delivery and recurrent ruptures of corpus luteum. The results of the coagulation tests showed obviously

prolonged APTT and TT, and slightly prolonged PT. And they could not be corrected by 1:1 normal plasma. On the other hand, the reptilase time was normal. The activities of coagulation Factors XII, XI, IX and X were lowered to $< 20\%$, but factors VIII and II were declined slightly. Protein C and S in plasma was 0% and 1%. Electrophoresis results showed a sub-peak of albumin and a slope $\alpha 1$ -globulin peak following a minor peak. Both of them were confirmed to have a T10038G heterozygous mutation in the $\alpha 1$ -AT gene of patients. Her 12-years-old daughter had no any bleeding syndrome, but showed abnormal coagulation tests as the proband.

Conclusion: The bleeding symptom of the patients with Pittsburgh is caused by the disbalance of inhibited coagulation and lower anticoagulation PC/PS, and for female patients, the great hemorrhage risk is valulation and delivery.

Disclosure of Interest: None declared.

PO534-WED

Congenital FXI deficiency: preliminary results of phenotypic (clinical and laboratory) and genotypic characterization of a case series from a single center

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Background: In congenital FXI deficiency there is a poor correlation between hemorrhagic symptoms and baseline FXI:Act.

Aims: Characterize the clinical and laboratory phenotype and genotype of FXI deficient patients, looking for a biological determinant of prognostic value for bleeding pattern.

Methods: Since September 2012 up to July 2014 we have prospectively evaluated 37 FXI deficient patients: 21F, 16M; median FXI:Act: 37% (range 0.5–57%); FXI:Act $\leq 1\%$ in 2 patients, $> 1 \leq 5\%$ in 6, $> 5 \leq 10$ in 3, $> 10 \leq 20$ in 2, $> 20 \leq 70$ in 24. A questionnaire has been administered to explore the clinical phenotype. These tests were performed: FXI:C, FVIII:C, VWF:Ag, VWF:RCO, AT, PC, PS, Omocystein, FII G20210A, FV Leiden, TAT, F1 + 2. Eighteen patients were evaluated for molecular biology.

Results: Sixteen patients were considered bleeders (median FXI:Act 18.5%), while 21 non bleeders (median FXI:Act 39%). No statistically significant differences were observed between the two groups as regard as results of all the above mentioned laboratory assays. Only 1 patient is positive for FII G20210A heterozygous and is a bleeder. Eighteen patients have been studied for molecular biology. Twelve showed a heterozygous mutation (missense or stop ones): 4/12 had compound heterozygous mutations; in 8/12 only 1 mutation was detected. Six/18 patients were homozygous. Exon 5 was involved in 8/18 patients; the G403T variation (type II), was the causative mutation in 6/8 patients. Exon 9 was involved in 7/18 cases. A mutation was found at intron 6, which involves a splicing site: it is reported that this variant causes no transcription of the whole exon 6 in the mRNA.

Conclusion: We confirm variability in bleeding phenotype among FXI deficient patients, not related to FXI:C levels. We could not find a biological determinant of prognostic value for bleeding pattern. Severe FXI deficient patients ($< 20\%$) present homozygous or double heterozygous mutations. Exons 5 and 9 are the most frequently involved. Prevalent mutations are missense or stop ones.

Disclosure of Interest: None declared.

PO535-WED

Factor VII deficiency and prophylaxis due to interventions

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Background: Factor VII deficiency is the most common of rare bleeding disorders. Its incidence is estimated at 1: 300,000–500,000. It is inherited in an autosomal recessive fashion and it affects men and women equally. Rare bleeding disorders are still undiagnosed in Macedonia. Two sisters with factor VII deficiency were diagnosed and registered for the first time in the Centre for haemophilia in 2014.

Aims: Awareness among the general population and health care professionals for rare bleeding disorders, especially for urgent and planned interventions.

Methods: The Centre for haemophilia has Laboratory for diagnosis of bleeding disorders. Laboratory diagnosis of factor VII deficiency is performed with one stage coagulation method on BCS-XP. In the Register of the Centre for haemophilia people with haemophilia and other bleeding disorders are registered. Recommendation for prophylaxis due to urgent and planned interventions is given to clinicians.

Results: One after the other, the two adult sisters with factor VII deficiency were diagnosed and registered in Centre for haemophilia in Macedonia 2014. Very soon after diagnosing, the first diagnosed sister had urgent curettage due to spontaneous abortion. Second sister had planned laparoscopic cholecystectomy. In two cases concentrate of recombinant factor VII^a was recommended as prophylaxis in dose of 15 µg kg⁻¹. In the first case prophylaxis was made with 2 doses and in the second case with 4 doses.

Conclusion: Diagnosing and registration of people with rare bleeding disorders in the haemophilia treatment centre is the first step toward an improvement of care for those people. Diagnosed people with rare bleeding disorders are treated adequately and prophylaxis is given due to urgent and planned interventions.

Disclosure of Interest: None declared.

PO536-WED

Course of pregnancy, labour and perioperative management of two patients with bernard-soulier syndrome – case reports and literature review

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Background: Bernard-Soulier syndrome (BSS) is a rare hereditary thrombocytopathy characterized by large platelets, thrombocytopenia of variable degree, and bleeding symptoms. The disease is caused by dysfunctional GPIb/IX/V heterocomplex or its absence.

Aims: We present the course of pregnancy, delivery and postpartum of two patients, one with grave BSS complicated by a history of DVT

and severe thrombocytopenia the other with mild BSS, PIH, swelling of the labia and urosepsis.

Methods: Observational analysis.

Results: 1. A 39-year-old patient with a history of chronic ITP was first diagnosed with Evans' syndrome. The patient experienced mucosal bleeding and a history of DVT. Immunological tests confirmed BSS diagnosis (decreased expression of GP Ib/IX by 58% and GP Ib – by 63.5%). The course of pregnancy was uneventful except for severe thrombocytopenia which required platelet, FFP and RBC transfusions, treatment with corticosteroids and IVIG. The c-section was performed at 37th week and the male fetus was born (2780 g/53 cm, Apgar 1st minute score -10). Despite extended treatment the postoperative period was complicated by wound and mucosal bleeds. 2. A 23-year-old patient without clinically overt thrombocytopenia underwent bone marrow assessment (megakaryocytes were present, no antibodies were detected). The patient was treated with corticosteroids and IVIG for 2 years with no effect and after trained volleyball. Immunological tests confirmed BSS diagnosis at the age of 20 (decreased expression of GP Ib/IX by 16% and GP Ib – by 28%). The course of pregnancy was complicated by rapidly progressing PIH. C-section was performed in 39th completed week and a female fetus was born (2980 g/ 50 cm, Apgar 1st minute score – 10). Postoperative course was complicated by PIH, massive swelling of the labia, urosepsis and SVT.

Conclusion: The two clinically different cases of BSS pregnant women highlights the need for establishing both hematological and obstetrical care standards for management of this rare disease.

Disclosure of Interest: None declared.

PO537-WED

Inherited FXIII deficiency in the northern region of Turkey

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Background: Factor XIII is a transglutaminase and stabilizing fibrin clot by strong covalent bonds between fibrin monomers. Umbilical cord bleeding, hematoma, and prolonged wound bleeding are the most frequent presentation. Factor XIII deficiency is an autosomal recessive disease and an extremely rare bleeding disorder with estimated incidence of 1 per 2-million population worldwide. Its prevalence in the northern Black Sea region of Turkey is unknown.

Aims: The aims of this study were to assess the prevalence of FXIII deficiency in the northern region Turkey in patients with coagulopathy.

Methods: This study used retrospective data collected from hematology laboratories and electronic medical records. The data included 1846 test results of FXIII assays that were performed between January 2005 and January 2015 at the University Hospital. This hospital is a tertiary referral centre of the central part of the Black Sea region of Turkey, which has a population of approximately five million people. Diagnosis of factor XIII deficiency was made by clot solubility test in 5M urea. Included patients have at least 3 repeated test positive.

Results: Inherited FXIII deficiency was detected in 16 of suspected patients. The median diagnoses age of the patients with FXI deficiency was 4.0 years (range, 1 month -43 years). There were 8 males and 8 females. There are three patients representing intracranial bleeding and one of them died. One patient has iliopsoas bleeding; one has deep muscle bleeding in femoral region. Patients were treated with fresh frozen plasma for FXIII substitution.

Conclusion: The count of FXIII deficiency is higher than expected ratio of our region population. High ratio of consanguineous marriages in region may contribute this prevalence. Prospective population screening, that includes DNA analysis, is recommended to determine the prevalence of inherited FXIII deficiency in this region.

Disclosure of Interest: None declared.

PO538-WED

Von willebrand disease: be or not to be, that is the questionAzevedo A¹, Freire I¹, Fidalgo T² and Gago T¹¹Clinical Pathology, Hospital São Francisco Xavier - Centro Hospitalar Lisboa Ocidental, Lisboa, Portugal; ²Clinical Hematology Departement/ Hemostasis Unit, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

Background: Bleeding disorders can be challenging to evaluate. Recent research has highlighted the clinical variability in bleeding symptom expression among defined bleeding problems. Moreover, risks for many bleeding disorders are unknown. Von Willebrand disease (vWD) is the most common inherited bleeding disorder, but variable severity and several classification types mean that diagnosis is often not straightforward.

Aims: Present the case of a patient with important hemorrhagic diathesis, whose diagnostic clarification has been very challenging.

Methods: Female, 30 years old, easy bruises, mainly after pregnancy 2 years before, diagnosis of vWD studied abroad. Determination of von Willebrand factor (vWF) levels, multimers analysis and molecular study and summary platelet aggregation study (PFA-100).

Results: Bleeding score 11/-3 to +45 (easy and severe bruising, wounds, dental extractions and menorrhagia), blood group 0, one eutocic delivery, no previous surgery, no hemorrhagic family history. Medicated with paroxetine, for depression. VWF levels (antigenic and functional) border-line, not < 30% (3 samples), normal multimeric distribution (2 samples), molecular analysis showed two missense mutations in exon 28, referenced in association with vWD type 2A and a splicing mutation in exon 38, not described. No deficits of clinically important factors (Factor XIII was not studied), no dysfibrinogenemia, normal platelets number and PFA-100 without changes.

Conclusion: Patient does not meet the criteria for the diagnosis of vWD. The slightly decrease of vWF levels by itself does not explain the severity of her bleeding. Factor XIII was not studied, because patient has no suggestive clinic. PFA-100 does not allow to detect all types of platelet changes. Thus, we have to consider platelet dysfunction (eventually enhanced by paroxetine?) and in order to clarify the situation complete studies of platelet aggregation and further study of the immediate family are ongoing.

Disclosure of Interest: None declared.

Regulation of coagulation and fibrinolysis II

PO539-WED

Identification of coagulation GENE 3'UTR variants that are potentially regulated by mirnasvan Hylckama Vlieg A¹, Martínez C², Vossen CY¹, Salloum-Asfar S², de Haan HG¹, Teruel-Montoya R², Corral J², Reitsma PH³ and Rosendaal FR^{1,3}¹Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands; ²Servicio de Hematología y Oncología Médica, Hospital Morales Meseguer. Centro Regional de Hemodonación, University of Murcia, IMIB-Arrixaca, Murcia, Spain; ³Eindhoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, Netherlands

Background: MiRNAs are recognized as critical regulators of gene expression, mainly through their interaction with the 3'UTR of their targets. Despite regulating different elements of hemostasis, their role in deep venous thrombosis (DVT) is still unknown.

Aims: To identify 3'UTR variants in coagulation genes that influence coagulation factor levels and DVT risk through miRNA modulation.

Methods: The 3'UTR of genes coding for FII, FVIII, FIX, FXI, fibrinogen, protein C, protein S, antithrombin, and TFPI were sequenced in subjects with extremely high or low levels of these factors in two case-control studies. Statistically predominant variants in one extreme level group were genotyped in 4485 cases/4889 controls, associated with plasma levels and DVT risk, and evaluated *in silico* and *in vitro* (luciferase assay) as potential targets of miRNAs.

Results: In total, 28 variants were identified. Five SNPs were predominantly present in one extreme level group (F2 rs1799963, F8 rs1050705 and F11 rs4253429, rs4253430, and rs1062547). Genotyping revealed that except the well known F2 rs1799963, none of these SNPs were associated with the risk of DVT. The three F11 SNPs were in high LD with functional variants rs2289252 and rs2036914. Analysis in controls wild-type for these known F11 variants showed that rs1062547 and rs4253430 (LD > 98%) provoked a significant increase of plasma FXI activity in heterozygotes (+7.6 IU mL⁻¹) and homozygotes (+8.5 IU mL⁻¹). *In silico* prediction revealed that these SNPs might disturb the binding sites of 2 miRNAs. Only miR-544 showed a significant decrease of the luciferase activity in comparison with a scrambled control (100% vs. 46 ± 9%, *P* = 0.029). This inhibition was not observed when using a mutated vector with rs4253430.

Conclusion: Two 3'UTR variants in F11 showed an effect on FXI plasma levels, which may be influenced in part by miRNAs. These results reinforce miRNAs as candidates to play a role in hemostasis and complex disorders such as thrombosis.

Disclosure of Interest: None declared.

PO540-WED

Identification of oestrogen responsive mirnas associated with the regulation of coagulation and the increased thrombotic risk in pregnant womenTay J¹, James I², Hughes Q³ and Baker R¹¹WACHT; ²Institute for Immunology and Infectious Diseases, Murdoch University, Murdoch, Australia; ³WA Centre for Thrombosis and Haemostasis, Murdoch University, Murdoch, Australia

Background: Plasma miRNAs are useful biomarkers but the role of miRNAs in coagulation is poorly studied. We reported that miR-494 is oestrogen responsive and directly inhibits *PROS1* expression, demonstrating an indirect mechanism for oestrogen-mediated acquired Protein S (PS) deficiency in pregnancy. We hypothesise that high oestrogen levels during pregnancy results in changes in the expression of a network of miRNAs which deregulate levels of haemostatic factors leading to increased thrombotic risk.

Aims: Use ncounter[®] miRNA assay for: 1. Generating miRNA expression profiles, 2. Identify control and oestrogen-responsive miRNAs from human plasma that target haemostatic factors associated with acquired PS deficiency in pregnant women.

Methods: Total RNA was extracted from plasma samples collected from nonpregnant females not taking (NP) or taking oral contraceptives (OC) and pregnant (PGT) women > 14 wks gestation. Four RNA samples from each cohort were analysed by ncounter[®] miRNA assay. From raw counts, pairwise comparisons of the cohorts were analysed by the quasi-likelihood method to identify differentially expressed miRNAs (*P* < 0.05) and NormFinder to identify stably expressed miRNAs. miRNAs of interest were further analysed by RT-qPCR and digital droplet PCR (DDPCR).

Results: Oestradiol levels in NP and PGT subjects were < 1 nM and > 25 nM, respectively. of 800 miRNAs assayed, 5 miRNAs were up-regulated and 8 miRNAs were downregulated in PGT compared to NP samples (*P* < 0.05). Eleven candidate control miRNAs were selected, of which miR-25-3p, miR-222-3p and miR-520f levels remained consistent in RT-qPCR and DDPCR assays.

Conclusion: We established a panel of endogenous control miRNAs suitable for studying changes in oestrogen-responsive plasma miRNAs, and 13 miRNAs showed significant changes with oestrogen compared to controls. Ongoing work is characterising the function of differentially expressed miRNAs in regulating coagulation and their potential role as biomarkers in pregnant women with high thrombotic risk.

Disclosure of Interest: None declared.

PO541-WED

Structural and functional studies of monoclonal high affinity antibodies (MABS) against the human TFPI kunitz-type protease inhibitor domain 1

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Background: Tissue Factor Pathway Inhibitor (TFPI) negatively regulates Tissue Factor (TF)-initiated coagulation through inhibition of both activated Factor X (FXa) and the TF/FVIIa complex. TFPI Kunitz domain 1 (K1) directly inhibits TF-bound FVIIa and contributes to the formation of the tight TFPI-FXa complex. Functional mAbs binding the N-terminal region of TFPI (TFPI (1-79)), containing the K1, but with different pro-coagulant potential were characterized.

Aims: Determine binding epitopes for inhibitory anti-TFPI mAbs all binding to TFPI (1-79), but affecting FXa binding differently - Identify the structural determinants for the different modes of FXa interaction

Methods: Binding interaction profiles were obtained for the FVIIa/soluble (s) TF complex, or FXa and TFPI by surface plasmon resonance (SPR) analysis. A TFPI alanine scanning library was used to generate binding profiles of the different mAbs by SPR. The anti-TFPI (1-79) Fab 2F22 and TFPI (1-79) complex structure was determined by X-ray crystallography.

Results: Although different mAbs competing for binding to TFPI all were inhibiting binding of sTF/FVIIa to TFPI, the effect observed on FXa binding differed. Some mAbs (e.g. mAb 2F22) reduced the affinity for FXa binding to TFPI, whereas others elicited no effect on this interaction. The Fab 2F22/TFPI (1-79) crystal structure identified a binding epitope comprising of residues preceding the K1 domain and several residues in the K1 domain of which Arg41, Arg65 and Glu67 seem to be particularly important, but the epitope does not include the P1 residue Lys36. Data generated using the TFPI alanine scanning library further substantiated the 2F22 epitope map. The data was used for a structure-function comparison of mAb 2F22 with mAbs that displayed less ability for reducing the FXa binding affinity to TFPI.

Conclusion: High affinity mAb binding to an epitope shown by 2F22 anti-TFPI (1-79) demonstrate blocking of FVIIa/TF inhibition but also reduction of the FXa affinity to TFPI.

Disclosure of Interest: A. Svensson Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, B. Kjær Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, J. Clausen Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, B. Krogh Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, P. Nørby Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, J. Breinholt Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S, H. Petersen Shareholder of: Novo Nordisk A/S, Employee of: Novo Nordisk A/S.

PO542-WED

ORM1: A Novel regulator of thrombin generation and potential cardiovascular risk factor

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Background: A recent GWAS analysis has shown an association between *ORM1*, a gene that encodes for $\alpha 1$ -acid glycoprotein ($\alpha 1$ -AGP), and the thrombin generation capacity of plasma. $\alpha 1$ -AGP is a transport protein in the blood that previous studies have reported to shorten the aPTT, and induce tissue factor (TF) expression in monocytes. The lead SNP in *ORM1* (rs150611042) has also been shown to have a strong cis-eQTL for *ORM1* expression in monocytes.

Aims: Here we explored these findings in MI patients and controls.

Methods: We genotyped rs150611042 in 196 patients who suffered an MI under the age of 50 years and 189 healthy age and gender matched controls. Thrombin generation was measured in citrated plasma from these subjects by Calibrated Automated Thrombogram (CAT) assay using the Stago PPP reagent (5 pm TF, 4uM PL), and TF was analysed in a chromogenic FXa generation assay (ADI). Levels of $\alpha 1$ -AGP were measured in plasma from patients ($n = 143$) and controls ($n = 150$) by ELISA. *ORM1* gene expression in monocytes was analysed in a separate cohort of MI patients ($n = 19$) and controls ($n = 19$).

Results: The *ORM1* rs150611042SNP was strongly associated with the lag time ($\beta = -0.539$ (SE 0.16); $P = 6.53 \times 10^{-4}$) but not with other measures of thrombin generation (ETP or peak thrombin), replicating our previous findings. Levels of $\alpha 1$ -AGP in the plasma were significantly higher ($P < 0.0001$) in MI patients (0.418 ± 0.01) vs controls (0.347 ± 0.01 mg mL⁻¹). Additionally, $\alpha 1$ -AGP levels showed a modest correlation with TF levels in both groups (Pearson $r = 0.285$, $P < 0.0001$) and a modest correlation with the ETP of the MI patients (Pearson $r = 0.3385$, $P < 0.001$), but not the controls. In a separate case-control MI cohort levels of *ORM1* mRNA in monocytes were expressed at higher levels in monocytes from the cases (1.78 fold increase, $P = 0.030$).

Conclusion: These findings support a role for $\alpha 1$ -AGP in regulating the rate of thrombin generation, and suggest that increased levels of this protein may be a risk factor for MI. The mechanism for this effect is unclear.

Disclosure of Interest: None declared.

PO543-WED

Role of coagulation factor XI in promoting distal platelet aggregation and thrombus formation in whole blood under flow

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Background: Coagulation factor XI (FXI) is a hemostatic serine protease that amplifies thrombin generation and inhibits fibrinolysis via thrombin activatable fibrinolysis inhibitor (TAFI). FXI contributes to experimental sepsis-associated platelet consumption. However, the mechanism by which FXI promotes local or distal platelet aggregation in circulation is ill-defined.

Aims: To evaluate FXI's role in local thrombus formation and distal platelet aggregation in flow.

Methods: Recalcified blood was perfused over surfaces coated with collagen +/- tissue factor (TF) and local platelet aggregation and fibrin formation was analyzed by light microscopy and Western blots. Downstream blood samples were analyzed for distal platelet aggregation by FACS. In select experiments, blood was exposed to microbial polyphosphates (long polyP) or treated with the anti-FXI mAb, 1A6, which selectively inhibits FXIa-mediated FIX activation without interfering with the hemostatic bypass activity of FXIa.

Results: Perfusion of blood over collagen +/- TF surfaces resulted in local platelet adhesion and fibrin formation that bound both thrombin and TAFI. Inhibition of FXI activity dramatically reduced local fibrin formation and thrombin and TAFI binding without affecting the extent of local platelet adhesion and aggregation. Presence of long polyP accelerated fibrin formation in a FXI-dependent manner. Robust platelet aggregation and eventual consumption was observed as a function of time in downstream samples. Inhibition of FXI activity significantly reduced the rate and extent of distal platelet activation and aggregation as well as the susceptibility of formed fibrin to tPA-induced lysis.

Conclusion: Targeted inhibition of FIX activation through the intrinsic pathway reduces collagen/TF-induced distal platelet aggregation without interfering with local platelet adhesion. This finding supports the rationale for selective targeting of FIX activation by FXIa as a potentially safe new antithrombotic approach.

Disclosure of Interest: None declared.

PO544-WED

Nucleic acids and polyphosphate as modulators of coagulation

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Background: Polyphosphate (polyP) and extracellular nucleic acids are implicated as mediating the connection between coagulation and inflammation. Though each are linear polymers of repeating phosphate units, they differ in charge spacing, flexibility, and propensity for secondary structure. Mechanisms by which these polymers modulate clotting reactions remain unclear.

Aims: Head-to-head comparison of the ability of nucleic acids vs. polyP to modulate clotting reactions.

Methods: Biotinylated polyP or nucleic acids were captured on streptavidin-coated plates, and their ability to bind thrombin was evaluated. *In vitro* clotting assays were used to determine the effect of these polyanions on clot formation and contact pathway activation.

Results: Long-chain polyP was substantially more potent at triggering the contact pathway than either RNA or DNA. PolyI and polyG RNAs (both of which form quadruplexes) were potent activators of the contact pathway, while other polynucleotide homopolymers had greatly reduced clotting activity. Oligonucleotides that can adopt stable quadruplex structures were far more effective at binding thrombin than were their linear counterparts, and thrombin bound with increased affinity to concatemerized quadruplex-forming units. Long-chain polyP was a more potent competitor of thrombin binding to DNA and RNA than short chain (platelet-sized) polyP.

Conclusion: Quadruplex structure formation was critical for nucleic acid interaction with thrombin indicating these structures may play an important role in extracellular nucleic acid mediation of coagulation and inflammation.

Disclosure of Interest: None declared.

PO545-WED

Selective neutralization of the serpin protease NEXIN-1 BY a specific monoclonal antibody

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Background: Protease nexin-1 (PN-1) is a serpin able to inhibit thrombin and enzymes of the plasminergic system. Such properties make PN-1 an interesting target for regulation of thrombus formation and dissolution.

Aims: We produced and characterized a neutralizing anti-PN-1 monoclonal antibody (MA-48H11).

Methods: The functional property of MA-48H11 was determined by measuring its effect on PN-1 inhibition of thrombin and plasmin amidolytic activities, towards their respective chromogenic substrates S-2238 and S1751. The effect of MA-48H11 was also analyzed on PN-1-thrombin and PN-1-plasmin interactions by SDS-PAGE. Moreover, fibrin clots performed in a purified system were used to measure the effect of MA-48H11 on the inhibition by PN-1 of both thrombin-catalyzed fibrin formation and plasmin-catalyzed fibrinolysis.

Results: As measured by the Biacore system, the K_a value for binding between MA-48H11 and human PN-1 was $4.7 \times 10^9 \text{ M}^{-1}$. The presence of a 20-fold molar excess of MA-48H11 over PN-1 did not prevent the inhibition of plasmin amidolytic activity by PN-1 whereas it significantly abolished the inhibition of thrombin amidolytic activity by PN-1 in a dose-dependent manner, with an IC_{50} of $2.2 \pm 0.5 \text{ nM}$. Thrombin/PN-1 complex formation was inhibited in the presence of MA-48H11, as evidenced by the disappearance of the thrombin/PN-1 complex on SDS-PAGE. In contrast, the plasmin/PN-1 complex was still formed in the presence of MA-48H11, showing that MA-48H11 did not affect the plasmin/PN-1 complex formation. MA-48H11 prevented by $95 \pm 6\%$ PN-1 activity toward clot formation induced by thrombin. In contrast, PN-1 inhibition of plasmin-induced clot lysis was totally insensitive to MA-48H11.

Conclusion: MA-48H11 is a blocking antibody that selectively neutralizes the anti-thrombin activity of PN-1 without affecting its anti-plasmin activity. This is the first time that a tool discloses an uncoupling of the antithrombotic and the antifibrinolytic properties of PN-1.

Disclosure of Interest: None declared.

PO546-WED

In silico prediction of microRNA binding sites in haemostatic factors

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Background: MicroRNAs (miRs) are small non-coding RNAs that regulate gene expression by binding the 3'UTR of target genes. A single miR can target multiple mRNAs, conversely single mRNAs may possess multiple miR binding sites; resulting in a large network of miR-mRNA interactions. The complex network, therefore, requires an efficient and targeted *in silico* approach in predicting miR interactions. We have shown oestrogen-mediated upregulation of miR-494, which directly binds the *PROS1*-3'UTR and decreases Protein S (PS) expression. However, miRs that target other haemostatic factors remain poorly characterised. Therefore, an *in silico* miR binding map for the coagulation pathways would greatly assist in prioritising miR candidate(s) selected for haemostasis studies.

Aims: Identify miR-mRNA regulatory networks in key haemostatic factors using multiple miR binding algorithms to create a weighted reference.

Methods: Three target prediction tools: TargetScan, miRNA.org and RegRNA were used to identify miR binding sites in the 3'UTRs of 40 haemostatic genes, and a list of predicted miR-mRNA interactions was compiled. The predicted interactions in the consolidated list were verified against published literature.

Results: of the 500 miRs predicted to bind to the 40 haemostatic genes, 235 bind to more than one haemostatic factor. For example, miR-19a, miR-19b, miR-27b, miR-181-b and miR-340 were predicted to bind > 8 genes, with tissue factor a common target amongst interactions. Moreover, predicted interactions of miR-19a, miR-143 and miR-29a with haemostatic factors, separately forecasted by 2 prediction algorithms, were corroborated by published literatures.

Conclusion: The compiled list of miRs and their predicted haemostatic targets is a useful reference for discovering miRs which target multiple haemostatic factors that are important in regulating haemostasis, and highlighting patterns of miR targeting across our genetic panel of haemostatic factors to prioritise miR candidates selected for future studies.

Disclosure of Interest: None declared.

PO547-WED

Analysis of the mechanism of species differences regarding recombinant human soluble thrombomodulin on protein c activation in human and mouse

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Background: ART-123, a recombinant human soluble thrombomodulin, exerts a pharmacological effect by promoting thrombin-mediated activation of protein C (PC), resulting in inhibition of thrombin generation (TG) by activated PC (APC). However, ART-123 does not exert that pharmacological effect strongly in rodent. Thus, current rodent models are limited in evaluating the properties of ART-123.

Aims: The objectives of this study were to investigate the mechanism why ART-123 does not exert a pharmacological effect through PC activation in rodents and to explore the experimental conditions under which ART-123 exerts a APC-mediated pharmacological effect in rodents.

Methods: An APC generation assay was used to investigate the effects of recombinant mouse soluble thrombomodulin (rmsTM) and ART-123 on mouse PC activation. Mouse thrombin clotting time (mTCT) was measured to analyse the potential of ART-123 to bind mouse thrombin, and a TG assay was used to explore the conditions under which ART-123 exerts an APC-mediated effect in mouse plasma.

Results: In the APC generation assay, although rmsTM and ART-123 promoted mouse thrombin-mediated mouse PC activation, the effect of ART-123 was weaker than rmsTM. In the mTCT assay, rmsTM and ART-123 prolonged TCT. In the TG assay, although rmsTM and ART-123 inhibited TG in mouse plasma, the effect of ART-123 was less potent than rmsTM. However, with an addition of human PC in mouse plasma, the inhibitory effect of ART-123 on TG was enhanced by human PC in a concentration-dependent manner, and ART-123 with human PC inhibited TG at comparable concentrations with rmsTM.

Conclusion: These results suggest that the difference in how ART-123 interacts with human PC and mouse PC plays a role in why ART-123 does not exert a pharmacological effect through PC activation in mice. Therefore, coexistence of ART-123 with human PC in mice models may be useful in the evaluation of the APC-mediated pharmacological effect of ART-123.

Disclosure of Interest: None declared.

PO548-WED

The immediate and late effects of thyroid hormone (TRIIODOTHYRONINE) on murine coagulation gene transcription

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Background: Thyroid dysfunction is associated with changes in coagulation.

Aims: To gain more insight in the role of thyroid hormone (TH) in coagulation control.

Methods: C57Black/6J mice received a low-iodine diet and drinking water supplemented with perchlorate to suppress endogenous triiodothyronine (T₃) and thyroxine (T₄) production. Impact of exogenous T₃ on plasma coagulation, and hepatic and vessel wall-associated (lung) coagulation gene transcription was studied in a short- and long-term.

Results: Comparing euthyroid conditions with hypothyroidism and replacement by incremental doses of T₃, dosages of 0 and 0.5 µg T₃/mouse/day were selected to study the impact of T₃ on coagulation gene transcription. A single T₃ injection increased hepatic transcript levels of the well-characterized T₃-responsive genes deiodinase type 1 and Spot14 within 4 h. This coincided with significantly reduced mRNA levels of fibrinogen-γ (*Fgg*), antithrombin (*Serpinc1*), protein C (*Proc*), protein Z (*Proz*) and protein Z-related protease inhibitor (*Serp10*), and the reduction of the latter three persisted upon daily T₃ treatment for 14 days. Prolonged T₃ treatment also induced a significant down-regulation in factor (*F*) 2, *F9* and *F10* transcript levels, while *F11* and *F12* levels increased. Activity levels in plasma largely paralleled these mRNA changes. Thrombomodulin (*Thbd*) transcript levels were significantly up-regulated after a single T₃ injection, which persisted upon prolonged T₃ exposure. Two-week T₃ administration also resulted in increased von Willebrand factor (*Vwf*) and tissue factor pathway inhibitor (*Tfpi*) mRNA levels, whereas tissue factor (*Tf*) levels decreased.

Conclusion: These data show that T₃ has profound effects on coagulation, with *Fgg*, *Serpinc1*, *Proc*, *Proz*, *Serp10* and *Thbd* responding rapidly, making these likely direct TH receptor targets. *F2*, *F9*, *F10*, *F11*, *F12*, *Vwf*, *Tf* and *Tfpi* are late responding genes and probably indirectly modulated by T₃.

Disclosure of Interest: None declared.

PO549-WED

Indirect regulation of hemostatic proteins by MIR24 AND MIR34A through hepatic nuclear factor 4 ALPHA (HNF4A)

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Background: MiRNAs have been described as CIS-regulatory elements of different hemostatic proteins, however their role as TRANS-regula-

tory elements mediated by a transcription factor is less known and could have a significant impact. HNF4a plays an essential regulatory role for multiple hepatic coagulation genes. *In vitro* studies have shown that *HNF4A* expression is regulated by miR34a and miR24, this latter predicted to bind to a conserved *HNF4A* region in mouse.

Aims: To analyze the consequences of *HNF4A* TRANS-regulation by miR34a and miR24 on several hemostatic proteins.

Methods: We transfected HepG2 cells and primary mouse hepatocytes (PMHs) with miR34a, miR24, and a negative control. We measured the expression levels of *F5*, *F8*, *F9*, *F10*, *F11*, *F12*, *SERPINC1*, *PROC*, *PROZ*, and *PROS1* by qRT-PCR. In human healthy livers (HHL; $n = 114$), we analysed transcripts levels of hemostatic proteins, as well as those of *HNF4A*, miR34a and miR24.

Results: HepG2 transfection with miR34a and miR24 caused significant reductions of *HNF4A*, *F11*, *SERPINC1*, *F10*, *F12*, *PROC*, and *PROS1* transcripts levels, while the latter four were only reduced in cells transfected with miR34a. MiR24 reduced significantly *HNF4A* as well as *SERPINC1* mRNA levels in PMHs. In HHL we observed significant and positive correlations between levels of *HNF4A* and *F5*, *F8*, *F9*, *F11*, *F12*, *SERPINC1*, *PROC*, and *PROS1* ($P < 0.0001$). Interestingly, in HHL samples with low (percentile 10) and high (percentile 90) expression levels of miR34a and miR24, we observed that *HNF4A* and all hemostatic factors were significantly and inversely correlated.

Conclusion: MiR24 and miR34a are two indirect regulatory elements of multiple hemostatic proteins. Variations in the expression of these miRNAs could explain in part differences of *HNF4A* expression levels and therefore levels of several hemostatic factors in healthy subjects. The clinical relevance of these findings on thrombotic or hemorrhagic disorders needs to be further investigated.

Disclosure of Interest: None declared.

PO550-WED

Investigation of mode of action of sugammadex effects on coagulation

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Background: The selective muscle relaxant binding agent sugammadex (Bridion®) is associated with short-lasting (≤ 30 min) and limited prolongation of activated partial thromboplastin time (APTT; 11%) and prothrombin time (PT; 17%) in humans. However, no sugammadex effects were observed on a standard thrombin generation test (TGT) which may be an additional explanation for the absence of increased bleeding risks when sugammadex is used in surgical patients¹. This however, requires identification of the mechanism by which sugammadex affects coagulation variables which is currently unknown.

Aims: The aim was to unravel the Mode of Action (MoA) by which sugammadex affects coagulation.

Methods: *In vitro* coagulation experiments were performed with plasma from healthy male volunteers spiked with sugammadex at concentrations corresponding with the mean peak plasma concentrations for the clinical doses of 2, 4 and 16 mg kg⁻¹ (25, 50 and 200 µg mL⁻¹, respectively). The experimental investigation included screening clotting assays, tissue factor-enriched TGT, Prothrombinase induced Clotting Time (PiCT) and factor Xa generation assays.

Results: Sugammadex concentration-dependently prolonged APTT and PT. At 200 µg mL⁻¹ the increase amounted to 10 s in APTT, 2.5 s in PT, and tissue factor-enriched TGT was delayed by 11–12 s. Sugammadex inhibited human factor Xa PiCT by approximately 1.7 s at 200 µg mL⁻¹, but not bovine factor Xa PiCT, suggesting species-dependence. Factor Xa generation demonstrated that sugammadex

specifically inhibits factor Xa generation via the intrinsic activation coagulation route. The effects of 200 µg mL⁻¹ sugammadex on APTT and PT correlated with an apparent reduction in factor X of 90% and 46% respectively.

Conclusion: It was concluded that the MoA by which sugammadex influences coagulation is likely by decreasing factor Xa activity and intrinsic activation of factor Xa formation. ¹ Anesthesiology, 2014 Nov;121(5):969–77.

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PO551-WED

Identifying substrates of protein disulfide isomerase using enzyme variants with altered redox potential

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Background: Inhibition of PDI blocks platelet accumulation and fibrin generation after vascular injury in an *in vivo* mouse models of thrombosis. The mechanism of PDI participation in thrombus formation is unknown. The two active sites of PDI include conserved CGHC motifs. Mutation of an active site cysteine can identify PDI reductase substrates. However this method is unable to detect those substrates requiring isomerization or oxidation.

Aims: We generated active PDI variants capable of reduction, oxidation, and isomerization of substrate proteins and capable of forming stable mixed disulfide complexes. Formation of disulfide complexes allowed identification of potential protein substrates of PDI.

Methods: PDI variants with alterations in the XX residues of the active site CXXC motif were expressed to identify PDI substrates undergoing reduction, oxidation or isomerization. PDI-associated proteins from platelets were isolated bound to the recombinant PDI mutants and identified by mass spectrometry. Protein substrates were confirmed by western blotting.

Results: Some of these substitutions resulted in active PDI variants with lower redox potentials and altered kinetics of enzyme-substrate interaction. These variants were able to reversibly transition between the reduced and oxidized state. Two PDI variants covalently linked to substrate proteins. Disulfide complexes were isolated and identified by mass spectrometry and confirmed by western blotting. Those substrate proteins that also bound to the inactive PDI were discounted. Platelet factor V and multimerin-1 were the only proteins identified which did not interact with the inactive PDI and were confirmed through blotting.

Conclusion: PDI is potentially responsible for reducing the disulfide-linked complex between platelet factor V and multimerin-1. The interactions between these three proteins and the required role PDI may play a critical role during the initiation of thrombus formation.

Disclosure of Interest: None declared.

PO552-WED

Inhibitory effect of vitamin D intake in plasma factor viii activity and tissue factor expression in monocytic cells

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Background: Reduced plasma vitamin D (VD) concentrations are associated with an increased risk of thrombosis in the general population, but more research is needed to clarify the mechanism underlying this clinical observation

Aims: To investigate VD effect in selected haemostatic factors.

Methods: We initially evaluated a three-arms cohort i.e. controls (C) ($n = 6$), deep vein thrombosis (DVT) patients ($n = 6$) and antiphospholipid syndrome (APS) ($n = 8$), with a follow-up period of 3 months after VD supplementation ($0.5 \mu\text{g week}^{-1}$). Peripheral blood was collected in citrate. The analysis was as follows: i) serum VD was evaluated by chemiluminescence ii) coagulation factors II, V, VIII, IX, X, XI, and XII were assayed by coagulometric methods, iii) TFPI and antithrombin activity were examined by chromogenic assays. Tissue factor (TF) expression was measured by qRT-PCR in immunoselected monocytes. Validation of FVIII results was performed in additional subjects, C ($n = 9$), DVT ($n = 6$), and APS ($n = 10$). Finally, THP1 cells were treated with either VD (100 nM) for 24 h, LPS ($1 \mu\text{g mL}^{-1}$) for 3 h or both and TF expression was evaluated

Results: Serum VD levels increased in all groups ($P < 0.05$), after VD intake. Among the coagulation factors evaluated, only FVIII plasma activity significantly decreased with VD supplementation in C (16%, $P < 0.01$), DVT (26%, $P < 0.01$), and APS group (29%, $P < 0.01$). Interestingly, TF mRNA was statistically lower after VD intake in DVT ($P < 0.01$), and lower in APS ($P = 0.08$) compared with the basal state. *In vitro* studies confirmed that VD consumption resulted in a down-regulation of TF expression in THP1, and significantly reduced the LPS-induced expression of TF

Conclusion: VD may contribute to an anticoagulant state by repressing FVIII activity in plasma and down-regulating TF factor expression in monocytes. In THP1, VD can repress TF expression in a normal situation. Indeed, a procoagulant state induced with LPS is counteracted by VD. Our results further explain the observed antithrombotic mechanism of VD

Disclosure of Interest: None declared.

PO553-WED

A Novel anti-tissue factor pathway inhibitor antibody, BAY 1093884, prevents bleeding in hemophilia a mice

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Background: BAY 1093884 is a fully human monoclonal antibody against tissue factor pathway inhibitor (TFPI) developed as a bypass agent for hemophilia patients with inhibitors. It restores thrombin burst for stable clot formation in hemophilic conditions *in vitro*.

Aims: The goal of this study was to elucidate the *in vivo* prophylactic hemostatic potency of BAY 1093884.

Methods: The hemophilia A mouse tail vein transection model was used for this study to mimic the venous bleeding characteristics of severe hemophilia. Male hemophilia A mice ($n = 12/\text{group}$) were treated prophylactically with escalating doses of BAY 1093884. All treatments were administered as single intravenous (IV) bolus at 4 different time courses prior to injury (1, 3, 5, and 7 days). Bleeding was induced by a 1.2-mm deep incision across the left lateral vein. Following injury, the animals were monitored hourly for moribundity for up to 9 h and after 24 h followed by euthanasia.

Results: Efficacious doses of BAY 1093884 providing 50% protection for survival (ED_{50}) for up to 6 days after IV bolus ranged from $0.6\text{--}2 \text{ mg kg}^{-1}$. In comparison, only 25% protection was achieved 6 days after the treatment with a very high dose of 1000 IU kg^{-1} of recombinant factor VIII (rFVIII). The effect of a single IV dose of 18 mg kg^{-1} BAY 1093884 providing 80%–90% survival was maintained over 8 days ($\text{ED}_{50} 6 \text{ mg kg}^{-1}$), whereas a single IV dose of rFVIII failed to provide protection over an 8-day period.

Conclusion: These studies demonstrate that BAY1093884 prevents bleeding and increases long-term survival to a greater degree than rFVIII in hemophilia A mice and may offer a convenient prophylactic treatment option for hemophilia patients with inhibitors.

Disclosure of Interest: M. Köllnberger Employee of: Bayer Pharma AG, P. Liu Employee of: Bayer HealthCare, D. Sim Employee of: Bayer HealthCare

PO554-WED

A novel anti-tissue factor pathway inhibitor antibody, BAY 1093884, restores hemostasis in induced hemophilia a rabbits with reduced thrombogenic potential

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Background: BAY 1093884 is a fully human monoclonal antibody against tissue factor pathway inhibitor developed as a bypass agent for hemophilia patients with inhibitors. It restores thrombin burst, leading to stable clot formation in hemophilic conditions *in vitro* and effectively stops bleeding *in vivo*.

Aims: Efficacy and thrombogenic potential of BAY 1093884 were evaluated in bleeding models in acquired hemophilia A rabbits and the Wessler venousstasis model, respectively.

Methods: In the Wessler model, thrombosis was induced by complete ligation of the jugular vein after administration of BAY 1093884, recombinant FVIIa (rFVIIa), or activated prothrombin complex concentrate (aPCC) to measure *in vivo* hypercoagulability. Anti-factor VIII antibodies rendered rabbits hemophilic, increasing bleeding time by ~3-fold (median 120–390 s) in an ear bleeding model and > 7-fold (median 240–1800 s) in a saphenous vein bleeding model.

Results: In both bleeding models, BAY 1093884 corrected bleeding time close to normal in a dose-dependent manner. A statistically significant reduction in saphenous vein bleeding was reached at 1 and 3 mg kg^{-1} BAY 1093884, reducing bleeding time to 330 and 240 s, respectively. In contrast, 1 mg kg^{-1} rFVIIa only slightly reduced bleeding time to a median of 1200 s. In the Wessler model, 1 mg kg^{-1} rFVIIa and 40 IU kg^{-1} aPCC resulted in thrombus formation, reaching full occlusion in rabbits with normal coagulation system. In contrast, BAY 1093884 up to 100 mg kg^{-1} did not fully occlude, and only showed a slight increase in localized clot formation over baseline, which could be completely prevented by an anticoagulant. In addition, no stasis-triggered thrombi were seen in hemophilia A rabbits treated with BAY 1093884, indicating reduced thrombogenicity risk for BAY 1093884 in patients with hemophilia.

Conclusion: These studies showed that BAY 1093884 potently controls bleeding in hemophilic rabbits while showing reduced thrombogenic risk compared with the current standard of care.

Disclosure of Interest: M. Köllnberger Employee of: Bayer Pharma AG, A. Trabandt Employee of: Bayer Pharma AG, V. Laux Employee of: Bayer Pharma AG.

PO555-WED

Antibodies to investigate the antithrombotic role of antithrombin IIIB

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Background: Antithrombin III β (ATIII β) is an isoform lacking the post-translational carbohydrate modification at Asn¹³⁵. This isoform binds heparin with greater affinity than ATIII α , and has been hypothesized to target antithrombotic function to the endothelial injury site.

Aims: To investigate the antithrombotic role of ATIII β .

Methods: We generated 2 monoclonal antibodies, TPP-2009 and A01, against the heparin-ATIII complex by phage display. Surface plasmon resonance (SPR) and functional assays were used to characterize the antibodies' modes of action.

Results: SPR studies showed that A01 binds both heparin-ATIII α and heparin-ATIII β whereas TPP-2009 is specific for ATIII β -heparin. In *in vitro* functional assays TPP-2009 partially but dose-dependently ablated the anticoagulant effect of heparin in both normal and hemophilia A human plasma when clot formation was triggered by factor Xa (FXa) or tissue factor. A01 completely inhibited heparin effects. In ATIII-deficient human plasma, TPP-2009 dose-dependently negated the effects of exogenously added ATIII β and heparin. In purified systems using ATIII β and pentasaccharide, TPP-2009 restored > 91% of FXa activity, confirming SPR data. TPP-2009 was tested in different species. TPP-2009 did not significantly ablate the effects of heparin in normal or hemophilic mouse plasma but dose-dependently reversed the effects of heparin in rabbit and cynomolgus monkey plasma. TPP-2009 was also effective in partially restoring FXa activity in rabbit and cynomolgus monkey plasma in the presence of FVIII-function-neutralizing antibodies. A01 completely restored FXa activity across all species tested.

Conclusion: We developed antibodies specifically against unique conformational epitopes on the complex of ATIII-heparin. TPP-2009 blocked ATIII β -mediated anticoagulation and effectively promoted thrombin generation in plasma. These results identify the importance of ATIII β in maintaining systemic hemostasis in plasma.

Disclosure of Interest: Y. Jin Employee of: Bayer HealthCare Pharmaceuticals, S. Yegneswaran Employee of: Bayer HealthCare Pharmaceuticals, P. Ramsey Employee of: Bayer HealthCare Pharmaceuticals, J.-M. Gu Employee of: Bayer HealthCare Pharmaceuticals, C. Patel Employee of: Bayer HealthCare Pharmaceuticals, F. Dittmer Employee of: Bayer HealthCare Pharmaceuticals, U. Gritzan Employee of: Bayer HealthCare Pharmaceuticals, M. Sherath Employee of: Bayer HealthCare Pharmaceuticals, L. Linden Employee of: Bayer HealthCare Pharmaceuticals, P. Bringmann Employee of: Bayer HealthCare Pharmaceuticals, V. Laux Employee of: Bayer HealthCare Pharmaceuticals, K. Kauser Employee of: Bayer HealthCare Pharmaceuticals, T. Hermiston Employee of: Bayer HealthCare Pharmaceuticals.

PO556-WED

Polystat: a synthetic polymer hemostat for use in combination with factor replacement therapies for hemophilia A

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Background: Hemophilia A is treated by infusion of Factor VIII or the bypassing agent, recombinant Factor VIIa (FVIIa). Fibrin crosslinking, achieved experimentally by supratherapeutic levels of human Factor XIII, may also confer additional clot stability and protection from bleeding. We have developed an intravenous synthetic polymeric fibrin crosslinking hemostat (PolySTAT) that crosslinks fibrin protofibrils, increases clot stiffness, and provides resistance to fibrinolysis. We hypothesized PolySTAT could enhance the effect of FVIIa on clot formation in simulated Hemophilia A plasma and in hemophilic mouse blood.

Aims: Evaluate the combined effect of PolySTAT and FVIIa on viscoelastic clot formation in human Factor VIII-deficient plasma and Hemophilia-A mouse blood.

Methods: Tissue factor activated Rotational Thromboelastometry (ROTEM) was used to assess clot formation in FVIII-deficient human plasma and mouse whole blood treated with clinically-relevant concentrations of recombinant human activated FVIIa with and without fibrin crosslinking by PolySTAT.

Results: FVIII-deficient human plasma with 0.6–3.6 $\mu\text{g mL}^{-1}$ Factor VIIa and 250 $\mu\text{g mL}^{-1}$ PolySTAT had faster clotting onset times and 28–60% faster clot formation rates than plasma treated with FVIIa alone. Maximum clot firmness after combination treatment was increased 1.1–1.4-fold ($P < 0.05$). Addition of FVIIa and PolySTAT to hemophilic mouse blood also reduced clotting onset time, clotting rate, and increased clot firmness. PolySTAT combined with the lowest dose of FVIIa was functionally equivalent to clots formed with the highest dose of FVIIa alone.

Conclusion: PolySTAT used in combination with FVIIa enhanced clot formation in Factor VIII-deficient human plasma and mouse hemophilia A blood. Synthetic fibrin crosslinking materials may reduce the required dosages of factor concentrates required for management of hemophilic bleeding.

Disclosure of Interest: None declared.

Risk factors for venous thrombosis III

PO557-WED

The association of endogenous steroid hormone, SHBG, and precursor steroid levels with hemostatic factor levels in postmenopausal women

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Background: Exogenous estrogen/progestin use is associated with alterations in hemostatic factor levels but the relation between endogenous steroid hormones and hemostatic factor levels is incompletely characterized.

Aims: To evaluate the cross-sectional association between endogenous steroid and hemostatic factor levels among postmenopausal women.

Methods: In 131 postmenopausal women not using hormone therapy, we measured thrombin generation (TG), normalized activated protein C sensitivity ratio (nAPCsr), factor VII activity (FVIIc), antithrombin activity (ATc), and total protein S (PS) antigen levels. We measured estradiol (E2), estrone (E1), total testosterone (T), dehydroepiandrosterone-sulfate (DHEAS), DHEA, and androstenedione by mass spectrometry, SHBG by immunoassay, and calculated free T levels. Using multiple linear regression, we estimated mean differences in each hemostatic factor level associated with a 1-unit difference in each hormone, adjusted for age, BMI, smoking, and diabetes. Statistical significance was evaluated using the 0.00625 level within each hormone (0.05/8 hemostatic factors) and the 0.00069 level across all tests (0.05/72 tests).

Results: There was evidence that 1-unit higher levels of E2 (pg mL⁻¹), E1 (pg mL⁻¹), and DHEA (ng dL⁻¹) were associated with lower PS levels (%): E2 $\beta = -0.86\%$, $P = 0.002$; E1 $\beta = -0.23\%$, $P = 0.000073$; DHEA $\beta = -0.062\%$, $P = 0.005$. There was also evidence that 1-unit higher levels of DHEAS ($\mu\text{g mL}^{-1}$) were associated with lower TG peak (nM) ($\beta = -39.7$ nM, $P = 0.000019$), lower TG endogenous thrombin potential (ETP) (nMxMin) ($\beta = -143.1$ nMxMin, $P = 0.000020$), and lower nAPCsr (DHEAS $\beta = -0.89$, $P = 0.001$) levels. We found no evidence of other associations. After adjustment for the performance of 72 tests, evidence of the association of E1 with PS and of DHEA with TG peak and ETP persisted.

Conclusion: In cross-sectional analyses, higher E1 levels were associated with lower anticoagulant PS levels and higher DHEAS levels with less TG. Replication efforts in a larger sample size are warranted.

Disclosure of Interest: None declared.

PO558-WED

Obstructive sleep apnoea does not enhance hypercoagulability as measured by thrombin generation in the morbidly obese

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Background: Morbid obesity has been demonstrated to result in hypercoagulability measured by thrombin generation with increased peak thrombin and endogenous thrombin potential. Moreover there is increasing evidence for an association between sleep apnoea and cardiovascular risk.

Aims: We sought to determine whether hypercoagulability underlies this association in a cohort of patients with morbid obesity.

Methods: Consecutive morbidly obese individuals attending the obesity clinic at King's College Hospital were recruited into the study. Obstructive sleep apnoea (OSA) was defined using the oxygen desaturation index (ODI) the hourly average number of desaturation episodes ($\geq 4\%$ decrease in saturation from the average saturation in the preceding 120 s, and lasting > 10 s). Blood samples were drawn in the morning for haemostatic tests including thrombin generation (measured by calibrated automated thrombography), D-dimer (DD), factor VIII, fibrinogen and tissue factor pathway inhibitor (TFPI).

Results: Seventy six patients were recruited. Patients were stratified into three groups: no OSA [< 5 dips per hr.; $n = 34$], mild OSA [$5-15$ dips/h.; $n = 28$] and severe/moderate [> 15 dips/h.; $n = 14$]. There was no significant difference in DD, endogenous thrombin potential or peak thrombin between those with and without OSA. TFPI, thrombin generation lag time and fibrinogen were significantly raised in the severely apnoeic group only. Bivariate analysis showed positive correlations between ODI and lag time, TFPI, FVIII and fibrinogen; 0.322 ($P = 0.005$), 0.327 ($P = 0.004$), 0.228 ($P = 0.049$) and 0.306 ($P = 0.007$) respectively. Multivariate regression analysis adjusted for age, sex and ethnicity showed ODI to be an independent predictor of TFPI and lag time only.

Conclusion: This study suggests that hypercoagulability as measured by thrombin generation is not a major contributor to the association between OSA and cardiovascular risk in the morbidly obese.

Disclosure of Interest: None declared.

PO559-WED

Verema - an affinity proteomics study to identify and translate plasma biomarkers for venous thromboembolism

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Background: Affinity proteomics holds the potential to identify novel biomarker candidates. By using suspension bead arrays built on antibodies from the Human Protein Atlas (HPA) plasma samples are analysed for differential abundance of the targeted antigens.

Aims: Our aim was to apply a plasma proteomic profiling approach to determine candidate proteins that may improve current clinical tools for diagnosis and risk prediction in venous thromboembolism (VTE) and to further explore VTE pathophysiology.

Methods: Two case-control studies; the Swedish 'Venous thromboembolism biomarker study' (VEBIOS) and the Spanish 'Riesgo de Enfermedad Tromboembólica Venosa' (RETROVE) were analysed in the discovery stage. Only cases with first VTE, age 18–70 and free from malignancy were considered. All participants had signed informed consent and the studies were approved by the regional medical ethics committees. The selection of targets was based on evidence grade of association with VTE or intermediate traits, HPA antibodies availability and pre-set quality criteria. Plasma protein profiles were generated using two suspension beads arrays. Association with VTE was evaluated using linear regression model adjusting for age per sample set. The results from the two samples sets were merged assuming a fixed effect. Analytical replication was performed on citrated plasma together with repeated measure of EDTA plasma samples.

Results: In the discovery stage, 760 HPA antibodies targeting 410 proteins were selected for the analysis of 258 samples from the two studies. We found a limited set of targets associated with VTE including von Willebrand factor (Bonferroni-corrected P -value < 0.05). A diagnostic model using proteomic data only was generated. The lasso logistic model fitted to VEBIOS was performed well with AUC = 0.7 in RETROVE. The concordance between EDTA and citrate plasma was in average $\rho = 0.65$.

Conclusion: Our approach provided a good indicative tool to define potential candidates that were selected for further validation.

Disclosure of Interest: None declared.

PO560-WED

Analysis of prothrombin missense mutants at 596Arg by single nucleotide substitution for anticoagulant system

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Background: Prothrombin Yukuhashi mutation, a substitution of Arg for Leu at position 596 of prothrombin, has been reported as a cause of thrombophilia associated with antithrombin (AT) resistance. It has been also revealed that the mutant thrombin shows thrombomodulin (TM) resistance in terms of inhibition for fibrinogen clotting.

Aims: We assessed the influence of other missense mutations of prothrombin at 596Arg by single base substitution on natural anticoagulation system by AT and TM.

Methods: We prepared recombinant wild-type and mutant prothrombins, and evaluated their procoagulant activities. We analyzed inactivation profile by AT and thrombin-AT (TAT) complex formation for their active forms, thrombins. We next examined thrombin generation for reconstituted plasma mixing prothrombin-deficient plasma and each recombinant prothrombin. We also tested fibrinogen-clotting and activated protein C (APC) generation for all prothrombins in the presence or absence of recombinant TM (rTM).

Results: All recombinant prothrombins were applicably secreted except for 596Pro, and some of them decreased their procoagulant activities. After 30-min inactivation by AT, relative residual activity of the wild-type thrombin was $15 \pm 4.0\%$ ($n = 3$, mean $\pm$ SE), whereas those of all mutants were more than 80%. In the absence of heparin, all mutant thrombins formed only tiny amounts of TAT complex, whereas the wild-type formed large amounts of TAT in a time dependent manner. After 1-min incubation with rTM, relative fibrinogen clotting activity of the wild-type decreased to $16 \pm 0.12\%$, whereas those of all mutants were between 37% and 56%. In the presence of rTM, APC generation by all mutant thrombins were enhanced similarly to that by the wild-type.

Conclusion: All mutant thrombins caused by single nucleotide substitution at 596Arg possessed AT resistance as well as TM resistance in terms of fibrinogen clotting inhibition. 596Arg is an essential amino acid to regulate procoagulant activity of the prothrombin by AT and TM.

Disclosure of Interest: None declared.

PO561-WED

Red cell distribution width and future risk of venous thrombosis, myocardial infarction, stroke and cancer – a cause specific analysis

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Background: Growing evidence support an association between red cell distribution width (RDW) and risk of venous and arterial disease, and RDW has also been associated with cancer risk. Venous thromboembolism (VTE) is a known complication of cancer, and cancer might be associated with arterial disease. Moreover, growing evidence supports a bidirectional association between arterial and venous thromboembolic diseases. The cause-specific effect of RDW on VTE, MI, stroke and cancer is poorly studied.

Aims: To study whether the apparent associations between RDW and VTE, MI, stroke and cancer is inflicted by the reciprocal impact of these outcomes on each other.

Methods: RDW was measured in 24363 participants of the Tromsø Study in 1994–95. Incident VTE, MI, stroke and cancer were registered until December 31, 2010. Person-time was calculated from inclusion date to the date of the first occurring outcome, migration, death or end of the study period. Cox regression models with age as time scale were used to calculate conventional and cause-specific hazard ratios (HRs) for all outcomes. The analyses were stratified for sex, and adjusted for BMI, smoking, white blood cell count and hemoglobin. The regional committee of research ethics approved the study and all subjects gave informed written consent.

Results: During a median follow-up of 16 years, there were 502 first occurring VTEs, 1612 MIs, 956 strokes, and 2144 cancers. Men with RDW in the highest quartile had 71% (HR 1.71, 95% CI 1.09–2.67) higher risk of VTE than men in the lowest quartile. The risk estimate remained unchanged in cause-specific analyses (HR 1.74, 95% CI 1.01–3.00). RDW was associated with all outcomes in cause-specific analyses, and the magnitude of the risk estimates was essentially similar as for VTE.

Conclusion: Our findings imply that the associations between RDW and VTE, MI, stroke and cancer is independent of the sequence of events, suggesting that RDW is a common biomarker of these diseases.

Disclosure of Interest: None declared.

PO562-WED

Risk of venous thrombosis in persons with increased body mass index and interactions with other genetic and acquired risk factors: a case-control study

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Background: The high prevalence of obesity in industrialized countries has a substantial impact on venous thrombosis (VT) occurrence.

Prothrombotic gene variants like blood group non-O and factor V Leiden (FVL) are also prevalent in Caucasian populations. The combination of these prothrombotic risk factors with obesity may have a strong effect on VT risk, especially if these factors act synergistically.

Aims: Determine whether the presence of FVL with blood group non-O modifies the risk for VT in various body mass index (BMI) strata and in specific high VT risk subgroups.

Methods: In a population based case-control study, data on VT risk factors, BMI, blood group and FVL were available in 4062 VT patients and 4659 controls. Odds ratios (OR) for VT risk were estimated for BMI tertiles. The effects of BMI in different combinations with FVL and blood group non-O were evaluated. Subgroup analyses involved stratification by sex, VT type and location and presence of acquired risk factors.

Results: Compared with participants without FVL, blood group O and a BMI in the 1st tertile (reference group), an increase in BMI was associated with an increased VT risk: OR 1.9 (95%CI, 1.6–2.3) for those in the 3rd BMI tertile without FVL and blood group O. These ORs were 3.8 (95%CI, 3.2–4.6) in the 3rd BMI tertile of participants without FVL and with blood group non-O, and 5.4 (95%CI, 3.5–8.5) in the 3rd BMI tertile of participants with FVL and blood group O. When both FVL and blood group non-O were present, ORs were 9.1 (95%CI, 5.9–14.0) in the 1st tertile, 9.4 (95%CI, 6.6–13.5) in the 2nd tertile, and 12.5 (95%CI, 8.9–17.6) in the 3rd BMI tertile. Subgroup analyses revealed similar joint effects.

Conclusion: Individuals with a high BMI, blood group non-O and/or FVL are at high venous thrombosis risk. The findings were also present in high-risk subgroups and may have implications for targeted screening and thromboprophylaxis.

Disclosure of Interest: None declared.

PO563-WED

Dehydration as a possible cause of seasonal variations in the incidence of venous thromboembolism

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Background: Seasonal variations in the incidence of venous thromboembolism (VTE) were previously demonstrated, although the mechanism of this variability is not completely understood.

Aims: Our study was aimed to explore the correlation between seasonal variations in VTE incidence and dehydration.

Methods: In the present retrospective analysis, consecutive patients with proximal deep vein thrombosis (DVT) and/or pulmonary embolism (PE) diagnosed between January 2009 and December 2013 at the Rambam Health Care Campus were evaluated.

Results: The study population included 1496 patients, 48% men, mean age 63 ± 18 . Most VTE cases (82%) were provoked and 39% of patients had active cancer. The 4 months of peak incidence (3, 7, 10, 11) were compared with the 4 months of the lowest incidence (4, 5, 6, 12), showing a significant difference in VTE numbers (149 vs. 101 cases per year, $P < 0.001$). VTE occurred most frequently in November and least frequently in May ($P < 0.001$). In all subgroup analyses, including gender, provoked or unprovoked event and presence or absence of cancer, significant differences between the peak and lowest incidence months remained. In patients with unprovoked VTE ($n = 269$), serum levels of blood urea nitrogen (BUN) and hematocrit were significantly increased in the peak incidence group compared to the lowest incidence group (19.5 ± 12.3 mg dL⁻¹ vs. 16 ± 7 mg dL⁻¹, $P = 0.04$; $39.2 \pm 5.5\%$ vs. $37.4 \pm 5.8\%$, $P = 0.05$, respectively).

Conclusion: The current study has demonstrated that VTE occurrence exhibits a monthly variation that exists also in patients with provoked

events and even in those with cancer. Dehydration may be a potential explanation to the season-related variation in VTE incidence.

Disclosure of Interest: None declared.

PO564-WED

An affinity proteomics study for plasma biomarker candidates of cardiovascular disease in venous thromboembolism

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Background: There is an increased risk of subsequent cardiovascular events in patients with venous thromboembolism (VTE), however the underlying mechanism of this association is not fully understood. We previously employed an affinity proteomics profiling strategy using > 10,000 protein-specific antibodies from the Human Protein Atlas (HPA), which identified a panel of tentative plasma biomarker candidates for arterial cardiovascular disease (CVD), the 'VinnCardio' project.

Aims: Our aim was to explore the behaviour of the identified plasma proteomic profiles for arterial CVD-biomarkers in relation to VTE.

Methods: Two different study sets from the Swedish 'Venous thromboembolism biomarker study' (VEBIOS) were included in our preliminary study; 97 participants from the acute phase before diagnosis of VTE (49 VTE, 48 non-VTE), and 178 participants from an age- and sex matched case-control study with plasma samples taken after discontinuation of treatment. VEBIOS was approved by the regional medical ethics committee and all participants had signed informed consent. Plasma protein profiles were generated using a suspension beads array with antibodies from HPA. The association with VTE was tested using linear regression model adjusting for age per sample set.

Results: 380 HPA antibodies targeting 321 candidate proteins in the two sample sets of 275 individuals were analysed. A set of 6 proteins in independent pathways were found significantly associated with VTE at Bonferroni-corrected threshold of 0.05; one candidate in the acute set and further five in the post VTE set. One of our candidates is the *GP6*

platelet receptor involved in thrombosis, and for which a genetic polymorphism has been shown previously associated with VTE.

Conclusion: Our results indicated that a limited set of novel plasma biomarker candidates were shared between VTE and arterial CVD. These were selected for further validation using sandwich immunoassays.

Disclosure of Interest: None declared.

PO565-WED

Lung disorders in elderly people are associated with pulmonary embolism and not with deep vein thrombosis

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Background: Pulmonary embolism (PE) has long been thought to exclusively originate from a deep vein thrombosis (DVT), but recent studies suggest this may not always hold true in a middle-aged population with inflammatory lung disorders.

Aims: To study the association between lung disorders (chronic obstructive pulmonary disease – COPD, asthma and bronchitis) and venous thrombosis (VT) in a case-control study among elderly (> 70 years).

Methods: 401 Cases with a first-time VT and 431 controls without a history of VT were included in the two-center AT-AGE case-control study, performed in Leiden and Vermont. All subjects were ≥ 70 years. Information on lung diseases was obtained from questionnaires during home visits. We calculated odds ratios (OR) and 95% confidence intervals (95%CI) after adjusting for age, sex, study site and smoking status. Risk estimates were calculated after stratification for DVT and PE (+/-DVT). Informed consent was obtained and the study was approved by the Medical Ethical Committee of the Leiden University Medical Center.

Results: Individuals with COPD had a 1.8-fold increased risk of VT compared with individuals without COPD (95%CI: 1.1–2.9). The increased risk was limited to an increased risk of PE (OR 2.5, 95%CI: 1.4–4.3) while there was no association with DVT (OR 1.0, 95%CI: 0.5–2.1). Individuals with asthma only had a mildly increased risk of VT (OR 1.4, 95%CI: 0.7–2.7); again the risk was limited to PE: OR 1.7, 95%CI: 0.8–3.8; DVT: OR 1.0, 95%CI: 0.4–2.5. Bronchitis increased the risk of VT 1.5-fold (95%CI: 0.9–2.3), with similar risk estimates for DVT and PE: 1.4 (95%CI: 0.8–2.6) for DVT and 1.6 (95%CI: 0.9–2.7) for PE. When analysing the risk of VT for all lung diseases combined, we found a 1.7-fold increased risk (95%CI: 1.2–2.4) for all VT; 2.1-fold (95%CI: 1.4–3.1) for PE and 1.2-fold (95%CI: 0.8–1.9) for DVT.

Conclusion: Inflammatory lung disorders in elderly individuals, i.e. COPD, asthma, bronchitis, are associated with an increased risk of PE but not, or only very mildly, for DVT.

Disclosure of Interest: None declared.

PO566-WED

Assessment of risk factors in established cases of deep vein thrombosis (DVT) and great saphenous vein thrombosis (GSVT) in Indian patients

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Background: Limited details available from India on the incidence and risk factors in the established cases of DVT & GSVT.**Aims:** A prospective study designed among the hospitalized patients with proven DVT and GSVT to assess the incidence and risk factors among Indian patients.**Methods:** Institutional Ethics committee approval taken & patients with proven DVT & GSVT for the period August 2013-December 2014, at the Southern Railway Headquarter Hospital, Chennai evaluated with a questionnaire. Patients with primary cause were evaluated with Hypercoagulability profile workup (APLA [Anti-phospholipid Antibody] Ig G & Ig M, Factor III, Protein C & S deficiency, Serum Homocysteine)**Results:** 85 patients of established DVT and 16 patients of GSVT evaluated. Majority of DVT patients were Males ($n = 57$) and GSVT patients were females ($n = 10$). [Median age: 55 years DVT group & Median age: 43 years GSVT]. Most common location of DVT was femoropopliteal (49%) followed by iliofemoral site (28%). Primary causes more commonly encountered in both the groups. Secondary causes included, surgery within last 12 weeks (37.8%), followed by cardiovascular accidents in last 12 weeks (18.9%) followed by patients on anticancer therapy (16.2%). Hyperhomocystenemia was the most common coagulation anomaly followed by protein S deficiency. Majority of DVT patients (65.8%) received unfractionated heparin.**Conclusion:** Hyperhomocystenemia is the most common coagulation anomaly in the study population. A larger multicentric study on its role in DVT/GSVT among Indian patients is required. Local guideline for management of superficial venous thrombosis is warranted.**Disclosure of Interest:** None declared.

PO567-WED

Joint effect homozygous carriers of a risk allele in the fibrinogen gamma chain and overweight on risk of venous thromboembolismLønneberg T^{1,2}, Smith E^{3,4}, Brækkan S^{1,2}, Horvei LD^{1,2}, Solomon T⁵, Wilsaard T⁶, Rosendaal F⁷, Frazer K^{3,4} and Hansen J-B^{1,2}¹K. G. Jebsen – Thrombosis Research and Expertise Center ^{TREC}, Department of Clinical Medicine, UiT - The Arctic University of Norway, Tromsø, Norway; ²Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway;³University of California, San Diego, La Jolla, CA, USA;⁴Department of Pediatrics, Rady's Children's Hospital, San Diego, CA, USA; ⁵Biomedical Sciences Graduate Program, University of California, USA;⁶Department of Community Medicine, UiT - The Arctic University of Norway, Tromsø, Norway; ⁷Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands**Background:** The prevalence of overweight is increasing and has become a major health challenge, among other reasons by being a strong risk factor for VTE. Fibrinogen is a key component of the hemostatic system. A risk allele in the fibrinogen gamma chain (rs2066865_A) (FGG) has robustly been associated with a moderate increase in VTE risk. No study has previously explored the joint effect of FGG and overweight on VTE-risk.**Aims:** To investigate the separate and joint effect of FGG and overweight on risk of VTE in a case-control study with participants recruited from the general population.**Methods:** Cases with incident VTE ($n = 613$) and age- and sex-matched controls ($n = 616$) were recruited among participants in the Tromsø study conducted in 1994-95 ($n = 27158$). DNA-samples were genotyped and body height and weight were measured at baseline. All VTE events were confirmed by radiological procedures or by autopsy. Overweight was defined as body mass index (BMI) ≥ 25 kg m⁻². The analysis was performed using logistic regression models by determining odds ratios (OR) for VTE in heterozygous-, homozygous- and non-carriers of FGG in normal-weight and overweight subjects. The analyses were adjusted for age and sex. The regional committees for research ethics in Tromsø approved the study, and all subjects gave their informed written consent.**Results:** The prevalence heterozygous- and homozygous carriers of FGG was 36.6% and 7.6%, respectively, and 59.5% were overweight. Overweight subjects without FGG had 40% higher VTE risk (OR 1.4; 95% CI 1.0–1.9) than normal weight subjects without risk alleles. Heterozygosity of FGG did not further increase the VTE risk in overweight subjects (OR 1.5; 95% CI 1.1–2.1), however, homozygous carriers of FGG had a 3-fold (OR 2.9; 95% CI 1.6–5.4) higher risk of VTE.**Conclusion:** Our findings imply that homozygosity of rs2066865_A in the FGG gene moderately increased the VTE risk in obese subjects.**Disclosure of Interest:** None declared.

PO568-WED

Impact of the ABO RS8176719 single nucleotide polymorphism on cancer-related venous thromboembolismDziewiecka O¹, Smith E^{2,3}, Brækkan SK^{1,4}, Jensvoll H^{1,4}, Blix K^{1,4}, Solomon T⁵, Wilsaard T⁶, Rosendaal FR⁷, Frazer KA^{2,3} and Hansen J-B^{1,4}¹K.G. Jebsen Thrombosis Research and Expertise Center ^{TREC}, Department of Clinical Medicine, UiT The Arctic University of Norway, Tromsø, Norway; ²Department of Pediatrics, Rady's Children's Hospital, San Diego, CA, USA; ³University of California, San Diego, La Jolla, USA; ⁴Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway; ⁵Biomedical Sciences Graduate Program, University of California, San Diego, La Jolla, USA; ⁶Department of Community Medicine, UiT The Arctic University of Norway, Tromsø, Norway; ⁷Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands**Background:** Cancer patients are at increased risk of venous thromboembolism (VTE). The ABO single nucleotide polymorphism (SNP) rs8176719 differentiates between O and non-O blood groups and variation at this site has been previously implicated in VTE. However, there is little information available on the role of this prothrombotic mutation in individuals with cancer.**Aims:** To study the joint effect of cancer and the ABO rs8176719 SNP on the risk of VTE in a case-cohort study recruited from the general population.**Methods:** Cases with a first VTE ($n = 612$) and an age-weighted sub-cohort ($n = 1668$) sampled from the Tromsø 4 (1994-95) and 6 (2007-08) studies were included. Incident cancer and VTEs were registered up to December 31st, 2012. Cox regression with adjustment for age and sex was used to determine risk of VTE in cancer compared to non-cancer subjects. Cancer was defined as an active cancer (i.e. 6 months prior to until 1 year following a cancer diagnosis). The study was approved by the regional research ethics committee and all subjects provided informed written consent.

Results: Genotyping was performed and the ABO rs8176719 risk allele (insG) was detected as heterozygous or homozygous in 1445 individuals. During a median follow-up period of 12.9 years, 520 developed cancer and there were 93 cancer-related VTEs. The risk of VTE in subjects with at least one risk allele was 3-fold (HR 3.1; 95%CI 2.4–3.9) higher than in those without the variant in cancer-free subjects. Cancer was associated with a 10-fold (HR 9.7; 95%CI 7.8–12.2) higher risk of VTE. The presence of the rs8176719 risk allele was associated with a 13.0-fold (HR; 95%CI 9.6–17.6) higher risk of cancer-related VTE and a 15.0-fold (HR; 95%CI 10.1–22.4) higher risk of cancer-related DVT. The ABO rs8176719 SNP explained up to 20% of cancer-related VTE events.

Conclusion: Our findings suggest that individuals with a non-O blood type are at moderately increased risk of cancer-related VTE.

Disclosure of Interest: None declared.

PO569-WED

Association between thrombomodulin C.1418C>T and venous thrombosis: a meta-analysis

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Background: Thrombomodulin(TM), a transmembrane glycoprotein that expressed on the surface of vascular endothelial surface, is an essential element in PC anticoagulant pathway. Studies in animals suggest that TM deficiency is associated with hypercoagulated state. Thrombomodulin c.1418C>T polymorphism is known to be a risk factor for arterial thrombosis such as coronary artery disease. However, the association with venous thrombosis still remains controversial.

Aims: To investigate the association between thrombomodulin c.1418C>T polymorphism and venous thrombosis.

Methods: Systematic searches of PUBMED, EMBASE, HuGE Navigator Genopedia database, Chinese Biomedical Database, Chinese National Knowledge Infrastructure, and WANFANG Database were performed. Pooled odds ratios(ORs) with 95% confidence intervals (95%CI) were calculated to assess the strength of the association. Subgroup analysis was conducted to seek for potential sources of heterogeneity. Sensitivity analysis was undertaken by removing an individual study each time to judge the reliability of the results. Publication bias was evaluated by funnel plot and Egger's test.

Results: A total of 7 studies were collected in our analysis, including 2379 cases and 5179 controls. No significant association between thrombomodulin c.1418C>T polymorphism and venous thrombosis was shown under the five genetic models (T vs C: OR = 1.05, 95% CI = 0.80–1.38; TT vs CC: OR = 0.90, 95%CI = 0.48–1.71; CT vs CC: OR = 1.08, 95%CI = 0.82–1.42; CT+TT vs CC: OR = 1.05, 95% CI = 0.80–1.39; TT vs CT+CC: OR = 0.86, 95%CI = 0.49–1.52) and the heterogeneity between studies was evidently revealed. Similar results were observed in the following subgroup analysis based on ethnicity, control source and study type.

Conclusion: Current studies on thrombomodulin c.1418C>T polymorphism are of great heterogeneity. It might not be a risk factor for venous thrombosis.

Disclosure of Interest: None declared.

PO570-WED

Neutrophils and factor XII, unlike platelets, are not crucial for development of experimental spontaneous venous thrombosis

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Background: Recently, platelets, neutrophils, and factor XII have been described as being crucially required in a damage-free mouse model for initiation and propagation of venous thrombosis (von Brühl, JEM, 2012). Silencing anticoagulation in mice using synthetic small interfering (si) RNA targeting antithrombin and protein C results, without additional trigger, within 72 h in extensive thrombus formation in the head and fibrin deposition in the liver. This phenotype is fatal, but can be prevented by thrombin (Safdar et al., Blood, 2013).

Aims: To gain more insight in the role of platelets, neutrophils, and factor XII using this spontaneous thrombosis model.

Methods: Female C57B/6J mice were injected intravenous with siRNAs (5.75 mg/siRNA/kg) and monitored for development of the thrombotic phenotype when platelets (α -GPIb α), neutrophils (α -Ly6G), or factor XII (siRNA) were depleted.

Results: Depletion of platelets fully abrogated the thrombotic phenotype, both on macroscopic and microscopic level, after siRNA administration (0/8 vs 7/7 affected, $P < 0.001$). This was confirmed by significantly lower levels of liver fibrin deposition (9.5 vs 75.7 ng mg⁻¹, $P = 0.0013$). In the complete absence of circulating neutrophils, the onset, severity, and morphology of siRNA-triggered thrombosis remained unaltered (11/12 vs 8/12, $P = 0.31$), and there were no differences in liver fibrin deposition (51.9 vs 67.9 ng mg⁻¹, $P = 0.93$). Surprisingly, lowering plasma levels of factor XII (to 12.7% of control group) accelerated the onset of the thrombotic phenotype and mice were more severely affected (9/9 vs 4/10, $P = 0.01$), which was confirmed on a microscopic level.

Conclusion: In this model, development of thrombosis is dependent on thrombin and platelets (but not neutrophils) i.e. classical elements in thrombosis and hemostasis. Unexpectedly, factor XII appears to have a protective effect, which brings into question its proposed role in spontaneous thrombosis pathophysiology.

Disclosure of Interest: None declared.

PO571-WED

Endogenous thrombin potential is directly correlated with proteinuria severity and hypoalbuminemia in nephrotic syndrome patients

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Background: Nephrotic syndrome (NS) is characterized by massive proteinuria, hypoalbuminemia, and an increased risk for venous

thromboembolism (VTE). We recently reported that hypercoagulopathy is directly proportional to disease severity in NS animal models.

Aims: The aim of the present, IRB approved, study was to assess this relationship in human NS.

Methods: Prevalent and newly-diagnosed NS patients (7 children; 14 adults) from Nationwide Children's and Ohio State University, not taking anticoagulant or antiplatelet agents and with no history of VTE were enrolled. Platelet poor plasma was prepared from blood collected into 0.32% NaCitrate/1.45 μ M Corn Trypsin Inhibitor. Amidolytic plasma antithrombin activity (AT) and Endogenous Thrombin Potential (ETP) were determined and correlated to corresponding urinary protein:creatinine ratios (uPr) and plasma albumin ELISA (pAlb).

Results: The cohort exhibited a range of uPr values (median (range): 5.13 (0.1–45.4)). pAlb and uPr was directly proportional to ETP ($R^2 = 0.278$ & 0.191 , $P = 0.025$ & 0.07 , respectively). Some prevalent cases ($n = 7$) no longer had nephrotic-range uPr (≥ 2.0) at enrollment. In comparison to patients with nephrotic-range uPr ($n = 14$), ETP was lower ($P = 0.027$) and pAlb was higher ($P < 0.001$). Further analysis of the actively nephrotic group revealed that both uPr ($R^2 = 0.395$; $P = 0.016$) and pAlb ($R^2 = 0.310$; $P = 0.039$) were significantly correlated with decreasing AT. Interestingly, however, there was no correlation between AT and ETP.

Conclusion: These data suggest that disease severity in humans with NS is directly proportional to ETP, an established marker of thrombotic risk. Because TGA is not clinically available in many centers, further investigation of disease severity as a surrogate marker of thrombotic risk is warranted. Surprisingly, there appears to be no direct relationship between AT and ETP in NS. Thus, acquired AT deficiency may not fully explain NS-associated hypercoagulopathy.

Disclosure of Interest: None declared.

PO572-WED

Differential expression of mirnas in plasma and peripheral blood mononuclear cells collected from patients with unprovoked venous thromboembolism and healthy control individuals

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Background: MicroRNAs (miRNAs) has emerged as a new class of biomarkers in many cardiovascular diseases. However, little is known on the associations between miRNAs and risk of VTE.

Aims: To investigate whether miRNAs are expressed differently in plasma and peripheral blood mononuclear cells obtained from patients with unprovoked VTE compared to healthy controls.

Methods: We conducted a case-control study including 20 patients with a history of unprovoked VTE and 20 age- and sex-matched healthy control subjects recruited from the general population. The study was approved by the regional ethical committee and all participants gave their informed written consent. Total RNA was extracted separately from platelet-free plasma (PFP) and purified peripheral blood mononuclear cells (PBMCs). Profiling of 742 miRNAs identified in PFP was performed on the Universal RT microRNA PCR Human panels I and II following RNA isolation and reverse transcription. For normalization of the data, an average of the assays detected in all samples ($n = 40$) was applied. Expression analysis of 446 miRNAs identified in PBMCs was performed on the miRCURY LNATM microRNA array

following sample labeling and hybridization. Statistical analysis (paired t-test) was done to estimate the differences between the sample groups.

Results: Signals of high quality from 97 miRNAs were detected in PFP-samples. Of these, miR-10b-5p, -320a, -320b, -424-5p, and -423-5p were upregulated, whereas miR-103a-3p, -191-5p, -301a-3p, and 199b-3p were downregulated in plasmas of VTE patients vs. controls ($P \leq 0.05$). Interestingly, the profiling of 446 miRNAs identified in PBMCs did not reveal any of the total number of miRNAs to be differentially expressed in the groups of VTE patients and controls.

Conclusion: Our findings indicate that plasma levels of nine miRNAs can be potential biomarkers for the diagnosis of unprovoked VTE. Whether these microRNAs have a causal role in VTE will be the subject for further research.

Disclosure of Interest: None declared.

PO573-WED

The risk of recurrent venous thrombosis in patients with (LOWER) leg cast immobilisation

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Background: People with (lower) leg cast immobilisation have a substantially increased risk of a first venous thrombosis. The effect on recurrent venous thrombosis is as yet unknown.

Aims: To estimate the risk of a recurrent event after (lower) leg cast immobilisation in patients with a history of venous thrombosis.

Methods: This study is a case-control study nested within a cohort of 4597 patients with a first venous thrombotic event who were followed over time for recurrence from 1999–2010 (MEGA follow-up study). Participants reported via a questionnaire on risk factors for thrombosis, including plaster cast in the first 3 months before a recurrence (cases) or in a random 3 month-period during follow-up for participants without recurrence (controls). In total 2723 (59%) participants returned the questionnaire. Odds ratios (OR), adjusted for age and sex were calculated to compare risk of recurrence between subjects with and without plaster cast.

Results: 2525 participants (93%) filled in information on plaster cast immobilisation. Twenty (1.0%) controls and 10 (2.2%) cases reported to have had below-knee plaster cast in the 3 months before control or recurrence date, for an OR of 2.5 (95%CI 1.1–5.4). We are currently evaluating and cross checking these data with these patients' medical records as well as collecting data on thromboprophylaxis use. Currently the data have been verified in seven (0.3%) controls vs. six (1.3%) cases leading to an odds ratio of 4.6 (95% CI: 1.5–14.2).

Conclusion: Below-knee cast immobilisation increases the risk of recurrent venous thrombosis in the 3 months after its application.

Disclosure of Interest: None declared.

PO574-WED

The prevalence of congenital thrombophilia in children treated for acute lymphoblastic leukemia with thrombosis

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Background: The thromboembolic complications are observed among children treated for acute lymphoblastic leukemia.

Aims: The aim of the study was to evaluate factors predisposing to thrombosis, especially congenital thrombophilia, in children treated for acute lymphoblastic leukemia according to ALL IC BFM 2002 and 2009.

Methods: An analysis of 36 cases of thrombosis in 242 children (15%) treated due to ALL in the Department in years 2007–2013 was carried out. Age at onset, location, cause and treatment methods were taken into consideration. All analyzed patients underwent screening for congenital thrombophilia.

Results: The age of patients ranged from 1.5 to 17 years with the median of 8.5 years. Arterial thrombosis occurred in 2 persons (1%), venous thrombosis in 34 (99%) patients. Thromboembolism was usually related to the central venous catheter ($n = 31$, 91%). Massive thrombosis occurred in 7 patients (20%). Relapses occurred in 3 children. The most common causes of thrombosis were: the presence of the central catheter ($n = 31$; 91%) and L-asparaginase treatment ($n = 19$; 55%). The factor V Leiden mutation was found in 1 patient, protein C deficiency in 1 patient and increased level of factor VIII was detected in 4 cases. The mean concentrations of factor VIII, von Willebrand factor and antithrombin were not different in the groups with and without thrombosis. The concentration of fibrinogen measured after finishing the chemotherapy was similar in both groups.

Conclusion: 1. The treatment with L-asparaginase and the presence of central venous catheter are the main risk factors for thrombosis in children with ALL. 2. Screening for congenital thrombophilia in children treated for ALL should not be done routinely because the coexistence of this two conditions is rare.

Disclosure of Interest: None declared.

PO575-WED

The assessment of risk factors for thrombosis in children treated for non-hodgkin lymphoma

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Background: The association between non-Hodgkin lymphoma (NHL) and thrombosis is well established. Main causes of thrombus formation are neoplastic disease, central vein catheter and side effects of treatment with corticosteroids and L-asparaginase.

Aims: The aim of the study was to assess factors predisposing for thrombosis in group of children treated for NHL between 2007 and 2013.

Methods: Thirty one cases were analyzed. Age of onset of thrombosis, location, cause of the thrombosis and the anticoagulant treatment were assessed.

Results: Thrombo-embolic complications occurred in 6 children (19%). Age at onset of thrombosis ranged from 2 to 17.5 years, median 9.5 years. Arterial thrombosis occurred in 1 patient (17%) while venous thrombosis in 5 (83%) patients. Thrombosis occurred in 4 children with lymphoblastic lymphoma (LBL), in 1 child with large cell anaplastic lymphoma (LCAL) and 1 child with primary mediastinal B-cell lymphoma (PMBCL). Five of the 6 cases of thrombosis were related to central catheter (83%). Massive thrombosis occurred in 2 children (33%). The recurrent thrombosis was not observed. The most common causes of thrombosis were: the presence of the central catheter ($n = 5$; 83%) and the treatment with L-asparaginase ($n = 4$; 66%). Mutation of factor V Leiden gene was found in 1 patient. Two patients were treated with recombinant tissue plasminogen activator in systemic infusion (16%). Low molecular weight heparin used in 5 patients (83%) was changed to warfarin in 3 of them (50%). Bleeding complications of the treatment were not observed. All children showed total resolution of thrombosis.

Conclusion: 1. Central vein catheter is predominant location and cause of thrombosis in children with NHL. 2. Congenital thrombophilia is not additional risk factor for thrombosis in children with NHL

Disclosure of Interest: None declared.

PO576-WED

Genetic and acquired risk factors of venous thromboembolism in young patients in the north-west region of Russia

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Background: Inherited thrombophilia is a main predisposing condition of idiopathic venous thromboembolism (VTE). However the etiology of deep vein thrombosis (DVT) and pulmonary embolism (PE) remains unknown.

Aims: To identify the main acquired and hereditary risk factors of VTE in young patients and to find differences between the sex and clinical variants of VTE (DVT, PE).

Methods: 250 patients up to 45 years old with VTE (117 men and 133 women, mean age – 37.4 years (10–45)) who were genotyped by PCR-RFLP method. Acquired risk factors of VTE were considered according to sex of patients and clinical variants of VTE (DVT, PE). The differences in genotypes and acquired risk factors distributions were estimated by the Crosstabs procedure and Logistic regression with the definition of Fisher's exact test and χ^2 test, OR, 95% CI and $P < 0.05$.

Results: We found a statistically significant association with the risk of VTE in the presence of polymorphism factors FII (OR = 6.3; 95%CI: 1.9–21.4; $P = 0.0005$), FV Leiden (OR = 3.8; 95%CI: 1.7–95%CI: 1.0–2.6; $P = 0.056$). Significant differences have been detected between the sexes in the distribution of FXIII 34Leu / Leu (women vs. men, OR = 2.7; 95% CI: 1.2–6.1, $P = 0.023$) and EPCR (men vs. women, OR = 1.8; 95% CI: 1.0–3.3, $P = 0.042$). We didn't find any particular external factors provoking VTE in 48.8% of young patients. Significant risk factors of isolated DVT were as follows: the immobilization (X2, $P = 0.06$), injuries (X2, $P = 0.07$) and inheritance of FV Leiden (X2, $P = 0.103$). The main predisposing factors for DVT + PE were the viral infection (X2, $P = 0.05$) and the family history of VTE (X2, $P = 0.08$).

Conclusion: Our results may confirm an increased risk of VTE in young patients with mutations FII 20210 G/A and FV 1691 G/A, EPCR Ser219Gly, FI Ala312Ala, FI -455G / A and homozygous FXIII Leu34Leu (young women). There are differences in hereditary and acquired risk factors of VTE between the sexes and clinical variants of VTE (DVT or PE).

Disclosure of Interest: None declared.

PO577-WED

Lipid levels and risk of recurrent venous thrombosis: results from the mega follow-up study

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Background: Knowledge of risk factors for recurrent venous thrombosis (VT) is relevant, as it may influence decisions on the duration of

anticoagulation. Although lipids have been widely explored in first VT, data on their role in the risk of recurrence are scarce.

Aims: To assess whether lipids are associated with risk of recurrent VT, with particular attention to subgroup analyses, as patients with unprovoked events and men are at higher risk of recurrence.

Methods: Patients with a first VT were followed from their first event (1999–2010 MEGA follow-up study), and investigated in relation to levels of total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), non-HDL, triglycerides (TG), apolipoproteins A1 (apo A1) and B (apo B). Percentile (10, 25, 50, 75 and 90) cut-off points of lipid levels were established in controls from the MEGA Study. Consent and ethical approval were obtained for this study.

Results: Out of 2215 patients followed for a median of 7.4 years, 343 developed recurrent thrombosis (incidence rate 2.5/100 patient-years, 95%CI 2.3–2.8). There was no consistent association between lipid levels and overall recurrent VT across percentiles. In age and sex-adjusted models, the hazard ratios (HR) conferred by low levels of HDL and apo A1 (< 10th percentile compared with > 90th percentile) or high levels of TC, LDL, non-HDL, TG, and apo B (> 90th percentile compared with < 10th percentile), ranged from 0.80 (95%CI 0.49–1.29) to 1.55 (95%CI 0.90–2.68). Further adjustment for body mass index, estrogen-, statin use, and diabetes did not substantially change the HRs. Subgroup analyses for which we stratified by unprovoked and provoked events or by sex did not reveal an association with any of the lipids studied.

Conclusion: Lipid levels could not identify patients at an increased risk of recurrent VT in this study.

Disclosure of Interest: None declared.

Thrombophilia III

PO578-WED

Secondary thromboprophylaxis with rivaroxaban in major inherited thrombophilias

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Background: Rivaroxaban has been licensed for secondary prevention of venous thromboembolic disease in the UK since 2012 and its utility in patients with major thrombophilia has not been assessed separately although such patients must have been included in the original Einstein clinical trials.

Aims: The aim of this study was to assess the use of rivaroxaban in patients formally receiving long term vitamin K antagonists (VKA).

Methods: Data was collected on patients with inherited thrombophilias who were previously prescribed VKA for secondary venous thromboprophylaxis with a target INR of 2–3 and switched to rivaroxaban.

Results: Thirteen patients with known severe inherited thrombophilias proven by genetic analysis, were included (6 had antithrombin deficiency, 2 with protein S deficiency, 2 with protein C deficiency, 2 had Factor V Leiden homozygosity, 1 dysfibrinogenemia). The mean age was 40 (range 17–57). The follow up was 21 months (range 1–27 months). All were originally started on rivaroxaban 20 mg daily. Three of 6 fertile women in this cohort experienced menorrhagia, so were offered either restarting warfarin or have a dose reduction &/or were offered oral tranexamic acid 500 mg TDS: 2 patients required dose reduction to 10 mg OD and 1 to 15 mg OD, 2 required tranexamic acid. None of the patients wanted to return to VKAs. One female patient with antithrombin deficiency developed abnormal liver function tests on rivaroxaban, which normalised following cessation and became abnormal again after rechallenging with rivaroxaban. No

recurrent thromboembolic or major bleeds were seen. One teenager chose not to take rivaroxaban on days when she played football.

Conclusion: Rivaroxaban treatment was acceptable to all patients, and only stopped in one patient due to abnormal liver function tests. Menorrhagia was a side effect in some fertile women, but responded to dose reduction &/or treatment with tranexamic acid. None of those patients preferred to restart VKA treatment.

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PO579-WED

Monitoring drug activity of dabigatran and rivaroxaban by thrombin generation using the calibrated automated thrombinoscope

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Background: The new oral anticoagulants (NOAC), Dabigatran and Rivaroxaban, are not usually monitored. However, sometimes monitoring may be useful, but routine coagulation tests as prothrombin time (PT) or activated partial thromboplastin time (APTT) do not accurately reflect the drug effect. The thrombin generation test (TGT) may be capable of detecting the influence of small levels of coagulation and anticoagulation factors.

Aims: The aim of this study was to assess TGT in patients with NOACs and analyze its correlation with the usual hemostasis tests.

Methods: We studied 100 patients (Dabigatran 34, Rivaroxaban 66). Blood samples were collected in the peak (2 h after administration) and trough levels (12 h after administration for Dabigatran and 24 h for Rivaroxaban) of the drug. Routine PT and APTT were carried out. TGT was measured by a modified calibrated automated thrombogram (CAT, Thrombinoscope BV). Results are expressed as nM thrombin (height of curve). Statistical comparisons between the tests mentioned were performed using the paired t test and student t. Correlations were analyzed by Spearman test.

Results: Significant differences between peak and trough samples were observed. Rivaroxaban peak vs trough samples: PT, 26.1 vs 18.0 s; APTT, 38.0 vs 31.3 s ($P < 0.01$); Dabigatran peak vs trough samples: PT, 21.0 vs 18.7 s; APTT, 58.4 vs 48.8 s ($P < 0.01$). TGT showed greater differences between peak and trough samples than the routine coagulation test (Rivaroxaban: 51 vs 135 nM; Dabigatran: 89 vs 155 nM) ($P < 0.001$). TGT trough results were similar to normal patients. The TGT correlation was stronger with PT (Rivaroxaban: $\rho = -0.50$; Dabigatran: $\rho = -0.62$; $P < 0.001$) than with APTT (Rivaroxaban: $\rho = -0.42$; Dabigatran: $\rho = -0.41$; $P < 0.001$).

Conclusion: TGT is more sensitive for monitoring particular cases of NOACs than the general clotting tests PT and APTT, especially in the

trough samples. TGT can be useful to control patients receiving oral anticoagulant treatment with FIIa or FXa inhibitors.

Disclosure of Interest: None declared.

PO580-WED

Different patterns of thrombophilia markers in pregnancy complications

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Background: A significant number of pregnancy related disorders is associated to prothrombotic abnormalities, nevertheless the pathogenetic role of thrombosis in obstetric diseases and the efficacy of anti-thrombotic prophylaxis is still under debate.

Aims: To research differences in the behavior of the main thrombophilia markers in women with different pregnancy related disorders.

Methods: 97 consecutive non pregnant women have been enrolled within 2 years and divided in 4 groups according to the history of obstetric diseases: (i) 42 women with recurrent miscarriages; (ii) 17 women submitted to ovarian stimulation because of infertility, (iii) 19 women with pregnancy-related venous thrombosis; (iv) 19 women with other obstetric complications (IUGR, pre- or eclampsia, HELLP syndrome, fetal loss). The following thrombophilia markers were evaluated: gene mutations of F V Leiden and of prothrombin G20210A, Protein C, Protein S, Antithrombin (AT); antiphospholipid antibodies (APAs) (anticardiolipin, anti Beta2 GPI, Lupus anticoagulant).

Results: 1:5 women in all groups had APAs with similar prevalence, ranging from 23% in group (2) to 21.1% in groups (3,4). 31 out of 78 women (40%) in the (1,2,3) groups showed FV Leiden or prothrombin G20210A mutations with a FVLeiden/prothrombin G 20210A ratio of 2:1. Combined FV Leiden and prothrombin mutations were detected in one woman of group (3). In group (4) only a prothrombin G20210 A gene mutation was detected in one woman (5%). Congenital AT deficiency was diagnosed in two women respectively in groups (1) and (3). An association with APAs and hereditary thrombophilia markers was present in 3 women.

Conclusion: Inherited thrombophilia alone or in association with APAs may contribute to the development of recurrent miscarriages, infertility and pregnancy related venous thrombosis. In the other obstetric diseases APAs are the main thrombophilia markers involved in placenta-mediated pregnancy complications.

Disclosure of Interest: None declared.

PO581-WED

Beta2-glycoprotein I deficiency with venous thrombosis: identification and *in vitro* expression study of a missense mutation P.LYS38GLU in APOH gene

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Background: Venous thrombosis (VT) and its complications is a worldwide medical problem. Beta2-Glycoprotein I (β 2GPI) is a new found anticoagulant protein that Beta2-Glycoprotein I (β 2GPI) is a new found anticoagulant protein that can inhibit the procoagulation activities of thrombin and the platelet aggregation.

Aims: This study aimed to explore the specific mechanism of a mutation (p.Lys38Glu) in *APOH* gene to cause hereditary β 2GPI deficiency and thrombosis in a Chinese family.

Methods: The plasma level of β 2GPI was measured by ELISA and western blot, and anti- β 2GPI antibody was measured by ELISA. Lupus anticoagulant (LA) was assays using the dilute Russell viper venom time. Deficiency of the major natural anticoagulants, PC, PS, AT and TM, was excluded. Thrombin generation tests (TGT) of plasma were assayed by calibrated automated thrombography. A mutation analysis was performed by amplified and sequenced the *AOPH* gene. Wild type and mutant (c.112A>G) *APOH* cDNA expression plasmids were constructed and Functional assays were carried out.

Results: The thrombin generation capacity of the proband is relatively higher than other family members. Missense mutation p.Lys38Glu in *APOH* gene and LA was coexisted in the proband. The mutation leading to β 2GPI deficiency and thrombosis by impair the protein production/secretion and the platelet aggregation inhibitory function.

Conclusion: The recurrent thrombosis of the proband is associated with the coexistence of p.Lys38Glu in *APOH* gene and LA in plasma.

Disclosure of Interest: None declared.

PO582-WED

Towards identification of genetic risk factors in two dutch families with unexplained hereditary venous thromboembolism

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Background: Family studies have shown a strong heritability component for venous thromboembolism (VTE), but established genetic risk factors can be identified in approximately half of VTE patients.

Aims: To identify genetic risk factors in 2 Dutch families with unexplained hereditary VTE.

Methods: We performed whole exome sequencing in 10 affected relatives of two unrelated families with an unexplained tendency for VTE. We prioritized variants shared by all affected relatives from both families, and evaluated these in the remaining affected and unaffected individuals. We prioritized variants based on two different filter strategies: 1) candidate genes, and 2) very low allele frequency in the European-American and Dutch population. We then used whole exome sequencing data available from 96 unrelated VTE cases with a positive family history of VTE from the GIFT study to identify additional carriers and compared the risk-allele frequencies with the general population. Variants without any additional carrier were also retained for further analysis. Finally, we assessed the association of these variants with VTE in the population-based MEGA study (up to 4291 cases with VTE and 4866 controls).

Results: Six variants remained as putative disease-risk candidates. These variants are located in 6 genes spread among 3 different loci: 2p21 (PLEKHH2 c.3105T>C, LRPPRC rs372371276, SRBD1 rs34959371), 5q35.2 (UNC5A c.1869 + 23C>A), and 17q25.1 (GPRC5C rs142232982, RAB37 c.367-92G>A). In GIFT, additional carriers were identified only for the variants located in the 2p21 locus. In MEGA, additional carriers were identified in both cases and controls, without a difference in prevalence. In MEGA, no carrier of the UNC5A variant was found.

Conclusion: We were unable to identify novel thrombophilic defects with whole exome sequencing of two thrombophilic families. Overall, our study illustrates the complexity of identifying true associations for VTE by a whole exome sequencing approach.

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PO583-WED

Recurrent venous thromboembolism during pregnancy in women with quantitative antithrombin deficiency with or without antithrombotic prophylaxis

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Background: Little is known on pregnancies after an episode of venous thromboembolism (VTE) in women with quantitative antithrombin (AT) deficiency.

Aims: To investigate the frequency of pregnancy-related recurrent VTE and pregnancy outcome in women with AT deficiency.

Methods: Women referred (1986–2014) to the Thrombosis Center for thrombophilia screening after an episode of VTE who remained pregnant afterward were considered if they carried a quantitative inherited AT deficiency (antigen levels < 60%).

Results: Out of 50 women, 17 remained pregnant after VTE for a total of 29 pregnancies. Antithrombotic prophylaxis with enoxaparin 4000 IU or nadroparin 3800 IU od was prescribed in 16 pregnancies (by us in 14) of 11 women, whereas 13 pregnancies in 6 women occurred before the access to the Center and were not prophylaxed. Recurrent VTE complicated 1 pregnancy with antithrombotic prophylaxis (left popliteo-femoral, 10th week) and 3 pregnancies without (left popliteo-femoral, 10th week; left popliteal, 15th week; left popliteal 3rd day after a caesarean section) (6.3% and 23.1%, Fisher's exact test $P = 0.299$). Three other pregnancies without prophylaxis were complicated by superficial vein thrombosis. In women with and without prophylaxis the frequency of miscarriage and of late placenta-mediated obstetrical complications was similar (25 and 23%; 1.2 and 0%, respectively). The two groups of women with and without prophylaxis were similar in terms of median age at first VTE (24.0 and 22.4 y), parity (0.8 and 1.1), age at index pregnancy (33.7 and 32.3 y) and BMI (22.1 and 25 kg m⁻²).

Conclusion: In women with quantitative AT deficiency, 4000 or 3800 IU od of low-molecular-weight heparin reduces by 75% the frequency of recurrent pregnancy related VTE. The residual 6.3% of recurrent VTE despite prophylaxis may suggest the need of higher heparin doses. Obstetrical complications were similar in the two groups and in line with those observed in the general population.

Disclosure of Interest: None declared.

PO584-WED

Molecular analysis of proteins deficiency in thailand

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Background: Protein S (PS) deficiency is the most common thrombophilia found in Thai patients. Furthermore, persistently low PS levels are found in approximately 1% of healthy Thais.

Aims: To investigate the molecular basis of PS deficiency in both healthy subjects and thrombosis patients.

Methods: The subjects were 13 healthy persons who had persistently low PS and 31 patients who had thromboembolism with low PS. Free protein S (fPS) antigen levels were determined by ELISA with the normal cutoff of 50%. All 15 exons and the promoter region of *PROS1*

gene were PCR amplified and directly sequenced. Novel mutations were analyzed *in silico* using the PolyPhen-2 and DomPred for missense mutations and BDGP Neural Network for splice site mutations.

Results: Five (38.5%) of the healthy individuals were found to have detrimental mutations. Three mutations, p.R451*, p.Y560* and c.-168C>T, have been reported in Chinese (first two) and Japanese patients. A novel splice site mutation, c.1833 + 5G>C, was found in 2 healthy subjects: A 24-year-old female with total PS and fPS of 46% and 16.5% and a 75-year-old female with total PS and fPS of 66% and 24%, respectively. Ten (32.3%) of the patients with thrombosis were found to have harmful mutations. Four patients had the c.Y560* mutation that had been reported in China. Three of them were related. One patient each had c.-168C>T, p.D79Y and p.Q563* mutations that had been reported in Japan. Three had a novel mutation, p.D652Y. Two of them were brothers who had unprovoked deep vein thrombosis at the age of 37 with the fPS levels below 5%. The other one was a 23-year-old man who suffered from chronic thromboembolic pulmonary hypertension with the total PS and fPS levels of 36% and 18%, respectively.

Conclusion: The *PROS1* gene mutation profile in Thais overlaps with reports in other Asian countries. However, 2 novel potentially harmful mutations are discovered. Similar mutations are found both in healthy subjects and in patients supporting the multi-factorial pathogenesis of thromboembolism.

Disclosure of Interest: None declared.

PO585-WED

Inherited thrombophilia as a high risk factor of recurrent pregnancy loss in georgian population

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Background: Recently, inherited thrombophilia (Factor V Leiden (FVL), Prothrombin (PTH G20210A) and MTHFR (C677T) gene mutations) has been suggested as a possible cause of recurrent pregnancy loss (RPL). Inherited thrombophilia is a genetic disorder of blood coagulation resulting in a hypercoagulable state, which in turn can result in abnormal placentation.

Aims: According to this fact, that investigated mutations are differently distributed in populations, the aim of our study, which is firstly carried out in Georgia, was to determine the role of inherited thrombophilia in development of recurrent pregnancy loss in Georgian population.

Methods: 166 patients with RPL and 100 healthy controls (age 20–45) were genotyped by PCR.

Results: 152 women had miscarriages and 48-stillbirth out of 166 investigated patients. Among them, 34 patients had both pathologies. At least single mutation was detected in 106 cases (63.8%), while it was found only in 28% in control group. FVL hetero and MTHFR homozygous forms of mutations were significantly higher in patients than in control group (15.7/16.9% and 0/2% respectively). Different combinations of triple and double hetero and homozygous forms of FVL/PTH/MTHFR mutations (6 cases) were found only in patients. According to mutation carriage, no statistically significant difference was detected between patients with miscarriages and stillbirths. However, in the case of thrombophilia, positive correlation was found between advanced maternal age and pregnancy loss.

Conclusion: Since distribution and role of inherited thrombophilia in Georgian population has not been conclusively established yet, most women with RPL are not tested for these mutations. We confirm a significant role of inherited thrombophilia in development of recurrent

pregnancy loss (miscarriages and stillbirth), especially in advanced maternal age (< 30). We suggest genetic analyses to women with RPL. It might potentially prevent miscarriages and stillbirth, as serious pregnancy complications in Georgian Population.

Disclosure of Interest: None declared.

PO586-WED

Do endothelium-related genes and inherited thrombophilia interact in increasing pregnancy loss risk?

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Background: The development of placental vascular network requires a crosstalk between haemostatic and endothelial components. Growing evidence suggests that maternal gene variants may confer an unfavourable genetic burden able to influence pregnancy loss, either in early and late trimester. Clinical studies have investigated the relationship between inherited thrombophilia and pregnancy loss, with conflicting results, and few studies contemporarily investigating inherited thrombophilia and endothelial genes polymorphisms in pregnancy loss are available

Aims: To investigate, beyond inherited thrombophilia mutations, the role of endothelium-related genes, such as RAS and eNOS polymorphisms, in 450 women with history of unexplained pregnancy loss according to early and late events, compared with 300 women with uneventful pregnancy.

Methods: All subjects were genotyped for inherited thrombophilia and ACE, AGTR1, AGT, eNOS genes polymorphisms.

Results: Inherited thrombophilia and ACED allele were significantly and independently associated with fetal loss and interact in increasing pregnancy failure risk ($P = 0.0009$). By analyzing genetic markers according to gestational age and recurrence pregnancy failure, we observed that FII20210A was associated with both early (OR = 2.68, $P = 0.03$) and recurrent early event (OR = 3.34, $P = 0.02$), whereas ACED allele modulated pregnancy failure risk independently of gestational timing (early: OR = 1.62, $P = 0.04$; recurrence: OR = 2.17, $P = 0.02$; late: OR = 3.90 $P < 0.0001$). An interaction between inherited thrombophilia, and in particular FII20210A, and ACED variant in determining a six-fold ($P = 0.005$) and a four-fold ($P = 0.05$) increased risk of early and recurrent early fetal loss, respectively, was found.

Conclusion: ACED allele is able "per se" to modulate the risk of pregnancy failure independently of gestational age, increases the risk of both early and recurrent early pregnancy loss, and confers a four-fold risk of a late event in thrombophilic women

Disclosure of Interest: None declared.

PO587-WED

Rivaroxaban in long-term secondary prevention of VTE in patients with hereditary or acquired thrombophilia is as effective and safe as standard treatment with vitamin K antagonists

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Background: The ENSTEIN DVT and PE trials showed that the orally active direct FXa inhibitor rivaroxaban, is at least as effective and safe as VKA treatment for secondary prevention of Venous Thromboembolism (VTE). Only a limited number of patients with thrombophilia were included in these studies.

Aims: In a prospective pilot case control study performed in patients with hereditary thrombophilia and/or antiphospholipid syndrome (APLS) we compared the efficacy and safety of long-term treatment with rivaroxaban with the standard treatment with VKA.

Methods: *Thrombophilia group* : 28 patients with APLS (defined according to the ISTH criteria) who experienced at least one episode of VTE and 52 patients with hereditary thrombophilia (AT, PC, and PS deficiencies, FV Leiden and FII mutations) with at least two recurrent episodes of VTE. All cases received rivaroxaban 20 mg p.o. daily. *Control group*: consisted of 28 patients with APLS and 52 patients with hereditary thrombophilia on VKA at doses adjusted to target INR 2–3. Patients had regular consultation every 4 months and were assessed for thrombin generation using the CAT[®] assay.

Results: The follow-up period was 6 months to 2 years for both groups. Patients on VKA performed regular INR evaluation and dose adjustment. None of the patients in both groups experienced thrombotic nor hemorrhagic complication during the follow-up. Patients on rivaroxaban showed significant inhibition of thrombin generation measured at each consultation. The INR was within the therapeutic range in only 50% of cases.

Conclusion: Rivaroxaban 20 mg once daily was as effective and safe as VKA standard treatment for secondary prevention of VTE in this limited population of patients with hereditary thrombophilia and APLS. In such patients the clinical benefit of a long term administration of rivaroxaban without need for laboratory monitoring and any dose adjustment or food restriction, was highly appreciated and this strategy must be encouraged.

Disclosure of Interest: None declared.

PO588-WED

Obstetric history, course of pregnancy and labour of patients with mthfr mutation— case report and literature review

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Background: Metylenetetrahydrofolate reductase (MTHFR) enzyme is involved in the conversion of homocysteine to methionine amino acid. The effect of this mutation is the reduction of methionine concentration and increase in homocysteine plasma levels. This in turn can lead to hypercoagulation due to increased expression of tissue factor, impairment of protein C activation: impaired tissue plasminogen activator binding to endothelial cells and excessive activation of factor V. The risk increases with the presence of another mutation, pregnancy (leading abnormal pregnancy outcome), hormone administration, trauma or surgery.

Aims: To present a series of 30 patients with MTHFR mutation and their reproductive history based on 2 year experience of one center.

Methods: Prospective observational study.

Results: In a group of 30 women with newly diagnosed MTHFR mutation 5 cases were diagnosed with MTHFR C677T/T and 25 with heterozygotic mutation. The homocysteine level of all patients but one in each group was within normal range. Most patients had grave obstetrical history with intrauterine death and up to seven miscarriages (mean of 2 abortions per person). In the investigated group there were 6 ongoing pregnancies and 17 completed gestations with live-born infants. Obstetrical outcome was irrespective of the patient's history or family history. All patients who planned gestation after MTHFR-mutation was confirmed were included in standard prophylaxis. The fetal wellbeing was closely supervised by repeated ultrasound investigations, Doppler flow and non-stress tests. Labour induction was administered before term while c-section was performed on obstetrical indications.

Conclusion: The data from this series of women with MTHFR-mutation show that regardless of the normal homocysteine levels this group of patients with reproductive failure requires special medical attention. The review highlights the need for establishing both hematological and obstetrical care standards for management of these patients.

Disclosure of Interest: None declared.

PO589-WED

LMWH in prophylaxis of the repeated pregnancy complications in women with thrombophilia and multiple pregnancy after IVF

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Background: the IVF outcome depends on many factors, especially the quality of embryo. We observed the importance of thrombophilia screening in women undergoing IVF.

Aims: to evaluate the efficiency of anticoagulant, antioxidants and vitamins (folic acid, group B) in the course of preparation for IVF, in the IVF protocols, during multiple pregnancy and in the postnatal period.

Methods: the comparative analysis of 50 women with the physiological course of multiple pregnancy (I group) and at 50 women with patho-

logical course of pregnancy after IVF. Genetic thrombophilia (mutations in genes of factor V, prothrombin, fibrinogen), deficiency of protein C, antithrombin III, hyperhomocysteinemia and APA circulation were evaluated.

Results: in healthy women of I group thrombophilia was revealed in 7 women. In the II group 48 (96%) of women different forms of thrombophilia were found: factor V Leiden mutation – in 8 (16%), prothrombin gene G20210A – 6 (12%), hyperhomocysteinemia in 17 (34%), APS 22 (11%). The combined forms of thrombophilia (APA in combination with genetic defects and hyperhomocysteinemia) in 15%. Moderate preeclampsia developed in 34 (17%), placental abruption with antenatal death of both fetuses – in 5 women, placental abruption with antenatal death of 1 fetus – in 10 women, chorionic abruption at the early terms of pregnancy – in 10 women with multiple pregnancy after IVF. Premature birth at term 29–32 weeks took place in 15 women. Thromboses (ileofemoral, jugular veins) – in 2 women at the I trimester of pregnancy and in 2 women postnatally. 70 alive babies were born. Fetal losses were associated with antenatal death in 12 women (14 babies). Results showed the pathogenetic role of an initial thrombophilia in the development of complications of multiple pregnancy after IVF.

Conclusion: the conducted research allows to assume the necessity of application of anticoagulant, antioxidant therapy and vitamins from the fertile cycle and during all pregnancy.

Disclosure of Interest: None declared.

Tissue factor and factor VII - III

PO590-WED

Antibodies to the K2 and K3 domains of tissue factor pathway inhibitor (TFPI) differ in biodistribution and effect on plasma TFPI concentration

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Background: Tissue factor pathway inhibitor (TFPI) is an endogenous inhibitor of the extrinsic coagulation cascade. TFPI variably consists of 2 or 3 Kunitz-type protease inhibitor domains (K1, K2, and K3). The majority of TFPI available to the bloodstream is associated with the endothelium, and a minor fraction is present in plasma and in platelets.

Aims: To determine the drug biodistribution and the plasma TFPI concentration in rabbits treated with monoclonal antibodies (mAbs) that target the K2 or K3 domains of TFPI.

Methods: Rabbits received single doses of anti-TFPI mAbs. Plasma TFPI antigen was determined by ELISA. Biodistribution of anti-TFPI mAbs was studied by immunohistochemistry.

Results: Treatment with the anti-TFPI(K2) mAb was associated with a slow increase in plasma TFPI antigen, peaking 2–3 days after treatment. In contrast, treatment with the anti-TFPI(K3) mAb was associated with a fast increase in plasma TFPI antigen within hours after dosing, followed by a higher peak and longer duration of elevated plasma TFPI. The biodistribution of the anti-TFPI(K2) mAb in the skin was consistent with widespread binding to TFPI on the microvascular and lymphatic endothelium. In contrast, there was only minimal binding of the anti-TFPI(K3) mAb to endothelium.

Conclusion: The slow increase in plasma TFPI after treatment with the anti-TFPI(K2) mAb suggests that the mAb extends the half-life of circulating TFPI, rather than affecting the secretion of TFPI into plasma. The fast increase in plasma TFPI after treatment with the anti-TFPI(K3) mAb suggests that a pool of TFPI is liberated from the endothelium. The different biodistributions of the anti-TFPI(K2) and anti-TFPI(K3) mAbs suggest that TFPI isoforms containing the K3 domain are either much less available on or not stably associated with

the endothelial cell surface. The pharmacodynamics and biodistribution of the anti-TFPI antibodies seen in these animals is supportive of further development of anti-TFPI therapies for patients with haemophilia.

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PO591-WED

Exploiting the biology of tissue factor pathway inhibitor: prospect for future therapeutic targets

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Background: TFPI is a serine protease inhibitor and is the only known regulator of the TF-dependent pathway of blood coagulation. Tissue factor (TF) - initiated coagulation has been implicated in the pathophysiology of many diseases including cancer and inflammation

Aims: This review is aimed at assessing the molecular biology of TFPI and its potential relevance for future therapeutic targets.

Methods: The PUBMED, EMBASE, MEDLINE and the Cochrane Database of systematic reviews were searched for relevant English-language reports of clinical trials, studies and abstracts on the biology of TFPI and its therapeutic implications. Congress Web sites were also consulted.

Results: Showed two isoforms of TFPI have been identified. TFPI α and TFPI β arise from an alternative splicing event and are differentially regulated at translational level. The two isoforms are expressed in different cell types and are optimized for different anticoagulant functions. TFPI α has 3 Kunitz-type inhibitor domains (K1, K2, and K3) and a basic C terminus, while TFPI β has the K1 and K2 domains attached to a glycosylphosphatidyl inositol-anchored C terminus. The GPI-anchoring of TFPI β allows it to optimally inhibit TF-FVIIa and FXa on endothelium. TFPI α needs the presence of Protein S as co-factor to bind to cell surfaces and optimally inhibit FXa, however PS is not required for FXa-dependent TF-FVIIa inhibition. The C-terminus of TFPI α enables it to inhibit early forms of prothrombinase, and its localization within platelet also facilitates this inhibition. Full length rTFPI has been found to be an effective antithrombotic agent in several clinical and animal studies. TFPI inhibitors are currently in development to treat bleeding disorders including hemophilia.

Conclusion: Our improved understanding of TFPI biology will enable us to better predict the molecular mechanisms of these inhibitors and further identify potential therapeutic avenues for treating bleeding and thrombotic disorders as well as other pathological conditions triggered by TF.

Disclosure of Interest: None declared.

PO592-WED

Population pharmacokinetic/pharmacodynamic modeling of a new recombinant human factor VIIa (LR769) to derive the dose to be studied in phase 3

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Background: Hemophilia A/B patients with inhibitors require a bypassing agent to treat or prevent bleedings. LR769 is an alternative recombinant human Factor VIIa under development. Classical dose-finding studies, assessing efficacy of a range of doses, are not feasible and therefore pharmacokinetic/pharmacodynamic (PK/PD) modeling was used to select a dosing regimen.

Aims: To derive a treatment regimen with a dose and interval of administration that is expected to be effective (best PD effects), providing potential cost efficiency (total drug needed) and patient convenience (number of administrations).

Methods: Data on the administration of 25, 75 and 225 $\mu\text{g kg}^{-1}$ LR769 to 15 hemophilia A/B patients (each received 2 doses) were used to develop a PK/PD model. IRB/EC approval was obtained. All patients provided informed consent. FVIIa activity was assessed by modified STACLOT rTF assay. PD tests used in the models: Thrombin generation (AUC of peak), aPTT, F1 + 2 and RoTEM+platelets+t-PA. Simulations of the PD effect of doses ranging from 75 up to 270 $\mu\text{g kg}^{-1}$ and variable intervals of administration were done.

Results: Concentration vs. effect relationship was determined for the PD parameters. Dosing regimens ranging from 75 to 100 $\mu\text{g kg}^{-1}$ provided similar maximum as well as trough effects with the exception of the 2 h dose interval which did result in a slightly higher PD effect at trough than 3 h. The trough PD effects of the 3 h dose intervals, however, were still significantly higher than baseline indicating continuing PD effects also 3 h after administration. No difference in PD effects at maximum or trough FVIIa concentration was seen between the dosages of 225 to 270 $\mu\text{g kg}^{-1}$.

Conclusion: Based on these results, two dosing regimens were chosen to be studied in the Phase 3 study: (i) 75 $\mu\text{g kg}^{-1}$ every 3 h and (ii) 225 $\mu\text{g kg}^{-1}$ once, if needed followed 9 h later by 75 $\mu\text{g kg}^{-1}$. Preliminary results of the ongoing Phase 3 study indicate these doses may be effective in the treatment of bleedings, indicating the value of PK/PD modeling.

Disclosure of Interest: J. Frieling Employee of: LFB USA, Inc., J. Stevens Employee of: CHDR, CRO contracted by LFB USA, J. Reijers Employee of: CHDR, CRO contracted by LFB USA, M. Moerland Employee of: CHDR, CRO contracted by LFB USA, J. Burggraaf Employee of: CHDR, CRO contracted by LFB USA, K. van Leuven Employee of: GBS, Lab contracted by LFB USA, C. Klufft Employee of: GBS, Lab contracted by LFB USA, J.-F. Schved Consultant for: LFB USA, Inc.

PO593-WED

Production of highly active recombinant human tissue factor pathway inhibitor

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Background: Tissue Factor Pathway Inhibitor (TFPI) inhibits Factor VIIa and Factor Xa through its Kunitz domains. Anti-TFPI strategies are developed in the treatment of bleeding disorders. We proposed a Gla-domainless factor Xa as a bait to TFPI in order to bypass a blocked tenase complex¹.

Aims: To further decipher the molecular interactions between TFPI and its partners, recombinant TFPI is required. This secreted protein, rich in disulfide bonds, is difficult to produce.

Methods: We investigated a series of expression systems in order to produce highly active TFPI alpha and set-up a purification procedure.

Results: Expression systems: 1-Bacterial systems: intracellular or *in vitro* expression systems were not successful because of truncation and improper disulfide bond formation. 2-Insect cells – baculovirus/Sf21 cells and drosophila S2 cells – were effective for production. The protein was secreted using its own signal sequence. The yield was 7 mg L⁻¹ in S2 cells after transient transfection and 1 mg L⁻¹ in baculovirus infected Sf21 cells. 3-HEK cells: the protein was efficiently secreted with a yield of 1 mg mL⁻¹. **Purification procedure:** When a C-terminal tag was added for purification, it was ineffective. We therefore produced native TFPI and purified the protein by impurities adsorption on Q-Sepharose, followed by chromatography on SP-Sepharose. Due to its high isoelectric point, the protein was eluted at 0.7M NaCl with a purity of 95%. **Quality control:** The purified proteins were analyzed by western blotting, functional ELISA, mass spectrometry (for determination of disulfide bonds) and functional assays (inhibition of factor Xa and VIIa *in vitro*).

Conclusion: The Drosophila S2 expression system was the most efficient and yielded TFPI that was able to stoichiometrically inhibit Factor Xa and Factor VIIa. This system can be further improved for higher yields.

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1-Marlu & Polack, Haematologica, 97, 1165-72 (2012).

Disclosure of Interest: None declared.

PO594-WED

Comprehensive assessment of the safety and efficacy of factor VIIa-CTP supporting phase 2A study

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Background: OPKO is a clinical stage public company developing bio-better long acting versions of existing therapeutic proteins utilizing a technology termed CTP. The technology involves fusion of the C terminus peptide of hCG to the target protein.

Aims: To evaluate the pharmacokinetic (PK), pharmacodynamic (PD), long term hemostatic effects of FVIIa-CTP and overall safety profile in mice, rabbits, rats and monkeys following IV administrations were supporting the ongoing phase 2 a study in hemophilic patients

Methods: FVII-CTP was expressed in CHO cells, purified and activated utilizing a CTP specific purification process. The toxicological effect, PK and PD of FVIIa-CTP was extensively characterized. In addition, the long term hemostatic effect was evaluated following bleeding challenge by tail vein transection (TVT) or tail clip assay as compared to commercial rFVIIa.

Results: The results of nonclinical pharmacokinetic studies conducted in mice, rats and monkeys consistently demonstrated a dose-proportional exposure and response and were significantly superior to those of rFVIIa, consistent with FVIIa-CTP prolonged half-life. In toxicological GLP studies FVIIa-CTP demonstrated an excellent safety profile without any unexpected adverse events at significant margins above the clinical doses. The thrombogenic effects was tested in Wessler model and was shown to be comparable to FVIIa. FVIIa-CTP had a profound effect on survival rate and reduced duration and intensity of bleeding as observed in tail clip study.

Conclusion: Attachments of CTP to FVIIa led to a pronounced enhanced PK and PD, elevated half-life and improved recovery supported by an excellent safety profile in toxicological setting. In addition, FVIIa-CTP resulted in an improved bioavailability translated to marked *in vivo* hemostatic effect. Our data suggest that CTP fused

FVIIa is safe, tolerable and has the potential to significantly improve the prophylactic and on-demand treatment of hemophilic patients.

Disclosure of Interest: None declared.

PO595-WED

Factor VIIa-CTP, a long-acting coagulation factor proposing a novel subcutaneous (SC) route of administration for prophylactic treatment – efficacy and safety in preparation for first in human study

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Background: OPKO Biologics is a clinical stage public company developing bio-better long acting versions of existing therapeutic proteins utilizing a technology termed CTP. The technology involves fusion of the C terminus peptide of hCG to target protein

Aims: The aim of this work was to comprehensively assess the feasibility of subcutaneous administration of FVIIa-CTP utilizing the most relevant *in-vivo* preclinical models in preparation for First in Human Study.

Methods: FVII-CTP was expressed in CHO cells, purified and activated utilizing a CTP specific purification process. FVIIa-CTP's pharmacokinetic (PK), pharmacodynamics (PD) and long term hemostatic effect and safety was extensively characterized following SC administration to rats, FVIII-/- mice, and pigs. In addition, utilizing the subcutaneous route, the long term hemostatic effect was evaluated following bleeding challenge as compared to commercial rFVIIa

Results: The studies demonstrated FVIIa-CTP provides long term exposure as reflected by AUC and half-life and significantly superior to those of rFVIIa, consistent with FVIIa-CTP prolonged half-life. In addition, an improved bioavailability was observed as compared to commercial FVIIa. Following SC administration, a profound improvement in clotting parameters, survival rate following TVT and reduced duration and intensity of bleeding in tail clip studies, were observed. Furthermore, FVIIa-CTP safety profile was further confirmed.

Conclusion: Attachments of CTP to FVIIa led to a pronounced enhanced PK and PD, increased exposure as reflected by AUC, elevated half-life and improved recovery in mice, rats and pigs following SC administration. FVIIa-CTP injected SC resulted in an improved bioavailability translated to marked *in vivo* hemostatic effect. Our data suggest that CTP fused FVIIa can potentially provide a novel approach for prophylactic treatment of hemophilic patients with the major benefit of significant improvement in quality of life.

Disclosure of Interest: None declared.

PO596-WED

The recombinant fusion protein linking activated factor VIIa to human albumin (RVIIA-FP) provides superior bleeding protection compared to recombinant FVIIa (RFVIIA) in a novel monkey model of acquired factor VIII inhibitors

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Background: The recombinant fusion protein comprising activated human coagulation factor VIIa linked to human albumin (rVIIa-FP) is characterized by a prolonged plasma and tissue half-life compared to recombinant factor VIIa (rFVIIa).

Aims: The aim of this study was to evaluate and compare rVIIa-FP and rFVIIa regarding their pharmacodynamic efficacy in a novel non-human primate (NHP) model of acquired hemophilia A with inhibitors.

Methods: Cynomolgus monkeys ($n = 4-5$ / group) were actively immunized with a subcutaneous administration of a recombinant, experimental human Factor VIII in the presence of the Th2-specific adjuvant, aluminium hydroxide. After a FVIII-mediated cross-reactivity of the resulting anti-drug-antibodies (ADA) to endogenous monkey derived FVIII lead to the development of clinical features closely mimicking the bleeding phenotype of patients with acquired hemophilia A. The extent of neutralizing antibody formation was monitored by measurements of anti-FVIII-antibody titers (including neutralizing antibody levels), FVIII activity and activated partial thromboplastin time (aPTT). Upon reaching a FVIII activity threshold level of $< 1\%$, animals received prophylactic treatment with either rVIIa-FP (1.5 mg kg^{-1}) or rFVIIa ($90 \mu\text{g kg}^{-1}$) at increasing dosing intervals. Effective prophylaxis was determined based on close monitoring of clinical observations, hematocrit (HCT) and hemoglobin (Hb) levels.

Results: The study confirmed effective prophylaxis of rVIIa-FP in a monkey model of acquired hemophilia A at dosing intervals of up to 8 h.

Conclusion: The study confirmed effective prophylaxis of rVIIa-FP in a novel monkey model of high clinical relevance. Direct comparison to rFVIIa suggested comparable efficacy of rVIIa-FP at reduced dosing frequency which would translate to improved therapeutic effectiveness.

Disclosure of Interest: E. Herzog Employee of: Employee of CSL Behring GmbH, E. Raquet Employee of: Employee of CSL Behring GmbH, H. Peil Employee of: Employee of CSL Behring GmbH, B. Doerr Employee of: Employee of CSL Behring GmbH, A. Veldman Employee of: Employee of CSL Behring GmbH, S. Schulte Employee of: Employee of CSL Behring GmbH, S. Zollner Employee of: Employee of CSL Behring GmbH, S. Zollner Employee of: Employee of CSL Behring AG.

PO597-WED

PF-05280602, a factor VIIa variant with enhanced *in vitro* potency compared to wild-type factor VIIa in hemophilic hemostasis assays

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Background: Recombinant activated wild-type Factor VIIa (WT rFVIIa) is used as bypass therapy for hemophilia patients with inhibitors but requires frequent dosing due to its short duration of action. PF-05280602 is a rationally designed variant of WT rFVIIa with improved potency and duration of effect *in vivo* in models of hemophilia.

Aims: To measure the *in vitro* potency of PF-05280602 in coagulation assays under hemophilic conditions.

Methods: Human hemophilia A and B plasmas dosed with PF-05280602 or WT rFVIIa were analyzed in the activated partial thromboplastin time (aPTT) based assay using Actin FSL (Siemens Healthcare) on a StarT 4 hemostasis analyzer (Diagnostics Stago) or thrombin generation assay (TGA) using PPP-Reagent Low (Diagnostics Stago) on a calibrated automated thrombogram system (Diagnostics Stago). PF-05280602 or WT rFVIIa was dosed into normal human whole blood treated with a Factor VIII neutralizing antibody (mAb 8015, Green Mountain Antibodies) and analyzed on thromboelastography (TEG) TEG5000 analyzers (Haemonetics) using Dade Innovin (Siemens Healthcare) and CaCl₂ (Siemens Healthcare) as a trigger.

Results: PF-05280602 exhibited a dose-dependent decrease in aPTT clotting time and normalized the clotting time at concentrations

approximately 6-fold lower than WT rFVIIa. PF-05280602 led to a greater reduction in the TGA lag time and increased endogenous thrombin potential and peak thrombin compared to WT-rFVIIa. Following Factor VIII neutralization in whole blood, PF-05280602 showed improvements in TEG parameters including clotting time (R) and clot formation time (K) over WT rFVIIa. Together, these studies demonstrate an increased potency of PF-05280602 compared to WT rFVIIa.

Conclusion: PF-05280602 demonstrated increased *in vitro* potency compared to WT rFVIIa in correcting hemostasis. PF-05280602 is currently in development as a therapy for hemophilia patients with inhibitors.

Disclosure of Interest: S. Patel-Hett Employee of: Pfizer Inc, S. Rakhe Employee of: Pfizer Inc, M. Zhao Employee of: Pfizer Inc, E. Madison Employee of: Catalyst Bioscience, S. Arkin Employee of: Pfizer Inc, J. Murphy Employee of: Pfizer Inc, D. Pittman Employee of: Pfizer Inc.

PO598-WED

Mathematical Modelling of membrane-dependent factor X activation by extrinsic tenase

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Background: The interaction of factors X and Xa with negatively-charged phospholipid membranes plays an essential part in the factor X activation by extrinsic tenase (VIIa-TF). Factors X and Xa can supposedly form oligomers on negatively-charged membranes, which additionally complicates the reaction mechanism.

Aims: Our aim was to create a mathematical model to investigate mechanisms of substrate delivery to extrinsic tenase, product removal and the role of oligomer formation.

Methods: The model was a set of ordinary differential equations based on a scheme of reactions including all possible stages of factor X activation. The system was solved using MATLAB.

Results: Factor Xa generation rate as a function of phospholipid concentration had distinctly different forms for different mechanisms of substrate delivery. The best agreement with experimental results was reached when only the membrane-bound factor X was used as a substrate. Product removal and oligomer formation had little qualitative effect on this function. However, oligomer formation essentially affected the dependence of factor Xa generation rate on factor X concentration. The velocity increased much faster in situation when oligomers interacted with tenase.

Conclusion: Mathematical model, which allowed differentiation between ways of substrate delivery, product removal and oligomers involvement, was designed. Predictions that allowed determination of the oligomers formation role were made.

Disclosure of Interest: None declared.

PO599-WED

In vitro mechanism of bay 1093884 inhibition of tissue factor pathway inhibitor

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Background: BAY1093884 is a fully human monoclonal antibody against tissue factor pathway inhibitor (TFPI) developed as a bypass agent for hemophilia patients with inhibitors. It restores insufficient thrombin burst, leading to stable clot formation in hemophilic conditions *in vitro*, and effectively stops bleeding *in vivo*. TFPI inhibits the

extrinsic tissue factor (TF)-initiated coagulation pathway. Full-length TFPI is composed of 3 Kunitz (K) domains (K1, K2, and K3).

Aims: To understand mechanistic differences of BAY1093884, which binds K1 and K2, and BAY1093889, which only binds K2

Methods: Both antibodies bind to human TFPI with high affinity (< 10 pM) and, by blocking TFPI function, have been shown to restore factor Xa (FXa) activity, promote thrombin generation, and shorten clotting time of hemophilic plasma and blood. We investigated the ability of the 2 antibodies to abrogate the TFPI-dependent inhibition of factor VIIa (FVIIa)-soluble TF (sTF) activity in purified component assays.

Results: In the presence of TFPI, both antibodies dose-dependently restored FXa activity of a chromogenic substrate. BAY1093884 (K1K2) but not BAY1093889 (K2) completely restored the activity of FVIIa-sTF toward a small substrate. This rescue of FVIIa-sTF activity by BAY1093884 was dose-dependent. Surface plasmon resonance (SPR) data indicate that BAY1093884 (K1K2), but not BAY1093889 (K2), competes with FVIIa-sTF complex for TFPI binding.

Conclusion: These results are consistent with the activity of BAY1093884 functioning through inhibition of both K1 and K2 domains of TFPI and preventing TFPI inhibition of both sTF-FVIIa and FXa. Conversely, BAY1093889 binds exclusively through the K2 domain of TFPI and exerts its effect by inhibiting TFPI binding to FXa. The kinetic data in conjunction with SPR provide differentiation in the mode of action between the 2 antibodies and the mechanistically novel mode of action of BAY1093884.

Disclosure of Interest: S. Yegneswaran Employee of: Bayer HealthCare, M. Köllnberger Employee of: Bayer Pharma AG, V. Evans Employee of: Bayer HealthCare, C. Patel Employee of: Bayer HealthCare.

PO600-WED

Evaluation of arioseven safety (recombinant activated factor VII) in patients with bleeding disorders (an observational post-marketing surveillance, study)

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Background: Recombinant activated factor VII induces hemostasis in patients with coagulopathy disorders. AryoSeven™ as a safe Iranian Recombinant activated factor VII has been available on our market.

Aims: This study was performed to establish the safety of AryoSeven on patients with coagulopathy disorder.

Methods: A single-center, descriptive, cross sectional study was carried out in Thrombus and Homeostasis Research Center Vali Asr Hospital during 2013–2014. Fifty one patients with bleeding disorders who received at least one dose of Aryoseven were enrolled. Patients' demographic data and adverse effect of drug and reaction related to Aryoseven or previous usage of Recombinant activated FVII were recorded in questionnaires. Finally data were analyzed to compare side effects of Aryoseven and other Recombinant activated FVII brands

Results: Aryoseven was prescribed for 51 Patients. of all participants with mean age 57.18 ± 21.38 years, 31 cases were male and 26 subjects had past history of Recombinant activated FVII usage. Glanzman was the most frequent disorder followed by congenital FVII deficiency, hemophilia with inhibitors, factor 5 deficiency, acquired hemophilia, hemophilia A with inhibitor, and hemophilia A or B with inhibitor. The majority of bleeding episodes had occurred in joints. Three patients (5.9%) complained about adverse effects of Aryoseven vs. 11.5% about adverse effects of other brands. However this difference was not significant, statistically.

Conclusion: Based on monitor patients closely for any adverse events, we concluded that Aryoseven administration under careful weighing of benefit vs. potential harm may comparable with other counterpart drugs.

Disclosure of Interest: None declared.

Transfusion II

PO601-WED

Dose-related pulmonary complication rates after fresh frozen plasma administration for warfarin reversal

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Background: Fresh frozen plasma (FFP) is administered to reverse warfarin anticoagulation. Administration has been associated with pulmonary complications but it is unclear whether this risk is dose-related.

Aims: We sought to characterize the incidence and dose relationship of pulmonary complications including transfusion-associated circulatory overload (TACO) and transfusion-related acute lung injury (TRALI) after FFP administration for warfarin reversal.

Methods: We retrospectively reviewed a cohort of patients on warfarin who received FFP for reversal over a 3-year period. Pulmonary complications were defined per the Center for Disease Control Hemovigilance Module and adjudicated by two independent clinicians and discrepancies resolved by a third clinician. Logistic regression was used to investigate the relationship between FFP dose and risk of pulmonary events.

Results: 255 patients were included. 149 (58%) were male and the median age was 76 years (range 23–96). Patients received warfarin for atrial fibrillation (64%), venous thromboembolism (25%), mechanical heart valves (8%), and other reasons (22%). Warfarin was reversed for clinically evident bleeding in 182 patients (71%) and surgery in 66 patients (26%). 49 patients (19%) developed pulmonary complications including 30 (12%) TACO, 2 TRALI (1%), and 17 (7%) pulmonary edema not meeting criteria for TACO. Pulmonary complication rate was significantly higher in those who received > 3 units of FFP (34.0% vs. 15.4%, $P = 0.002$). Stratified by subtype of complication, only TACO risk was significantly increased (28.3% vs. 7.4%, $P < 0.0001$). In multivariable analysis, > 3 units of FFP remained a significant risk factor for pulmonary complications (OR = 2.56, 95% CI 1.24–5.25, $P = 0.01$).

Conclusion: Almost 20% of patients who received FFP for warfarin reversal developed pulmonary complications, primarily TACO, and risk increased with > 3 units of FFP. Clinicians should be aware of and prepared to manage these complications.

Disclosure of Interest: None declared.

PO603-WED

Analysis of membrane microparticles and thrombin generation activity of DMSO cryopreserved platelets

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Background: Freezing was proposed as a promising approach to extended platelet (PLT) storage for transfusion medicine. The current state of the art method is 6% DMSO cryopreserved platelets (CPP).

Aims: To investigate platelet membrane changes in CPP, the size distribution of generated platelet membrane microparticles (PMPs) and their contribution to procoagulant activity of CPP products.

Methods: PMPs in CPP and liquid stored PLTs (LSP, paired control) were characterized by flow cytometry (FC), nanoparticle tracking analysis (NTA), confocal microscopy (CM), field electron microscopy (SEM, TEM), atomic force microscopy (AFM), thrombin generation (TG) assay, and light transmission platelet aggregometry (LTA).

Results: SEM and TEM showed marked disintegration and vesiculation of platelet plasma membrane, and loss of intracellular organization in CPP. FC counts of CD41a⁺ PMPs and annexin V-binding PMPs in CPP were about 20x and 70x higher, respectively, compared to LSP. Similarly, the NTA showed about 5x higher counts of PMPs compared to LSP processing control. NTA size distribution histogram of PMPs in CPP indicated a peak of about 100 nm of particle hydrodynamic diameter, corresponding to the size range of exosome-like vesicles. The TG assay showed that CPP possessed about 5x higher TG activity (thrombin peak height/10⁶ PLTs), compared to LSP. Interestingly, the exosome-like PMPs were responsible for most of the TG activity of CPP since the activity remained in the 20,000 g supernatant of CPP, but it was substantially decreased after spinning at 100,000 g.

Conclusion: The CPP exhibit dramatic platelet membrane vesiculation and release of exosome size PMPs responsible for a substantial part of procoagulant activity of the CPP product. Our results warrant further studies on phenotypes and activities of PMPs in CPP products. These findings and conclusions have not been formally disseminated by FDA and should not be construed to represent any Agency determination or policy.

Disclosure of Interest: None declared.

PO604-WED

Phosphatidylinositol-3-kinase is impaired following amotosalen and ultraviolet a light treatment of platelets in concentrates for transfusion

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Background: Photochemical treatment of platelet concentrates reduces chances of pathogen transmission. Amotosalen and ultraviolet A light illumination (AS-PCT) is currently used in several blood banks in Europe and was recently approved in the United States. We formerly published that AS-PCT significantly reduces thrombus formation on collagen under shear flow.

Aims: To elucidate the biomolecular mechanism causing this reduced thrombus formation.

Methods: Biochemical analysis of signal transduction pathways in paired AS-PCT treated or untreated platelets.

Results: AS-PCT treatment significantly impaired dose-response PAR1 mediated integrin $\alpha_{IIb}\beta_3$ activation measured by PAC1 binding. G_q and G_{12/13} signaling was unaffected because calcium entry, pleckstrin phosphorylation, shape change and degranulation were normal. We assumed that coupling to G_i was defective because $\alpha_{IIb}\beta_3$ activation by selective activation of P2Y₁₂ was decreased following AS-PCT. Because G_i activates phosphatidylinositol-3-kinase (PI3K), we measured Akt and found significantly less phosphorylated Akt in AS-PCT platelets in response to PAR1 activating peptide but also following c-MPL (thrombopoietin) and GPVI-FcγRIIIa activation. To determine if kinases/phosphatases acting on Akt were involved or rather the preceding PI3K, we indirectly measured phosphatidylinositol(3,4,5)trisphosphate (PIP3) formation through Bruton tyrosine kinase phosphorylation and found this to be significantly affected in AS-PCT platelets stimulated through GPVI-FcγRIIIa. Using lipidomics we showed that AS-PCT treatment causes covalent binding of amotosalen to unsaturated acyl side chains of multiple phospholipid species in platelets and speculate that this modification contributes to the observed phenotype.

Conclusion: We conclude that PIP3 formation is significantly impaired in AS-PCT platelets explaining the diminished thrombus formation under shear flow. Further research will determine if this defect is caused by the altered platelet plasma membrane phospholipid composition.

Disclosure of Interest: None declared.

PO605-WED

Inactivation of emerging viruses by pasteurization in plasma-derived medicinal products

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Background: Because some emerging viruses (eg, Chikungunya virus [CHIKV], Middle East respiratory syndrome coronavirus [MERS-CoV], dengue virus [DENV], and hepatitis E virus [HEV]) were transmitted by transfusion of blood or blood components, there is an ongoing concern for the safety of plasma-derived medicinal products (PDMPs) because of a potential virus load in the starting material plasma. These viruses might not be detected because donors may appear healthy during the disease incubation period, and screening to detect emerging viruses is not routine. Therefore, manufacturing processes of PDMPs must be able to inactivate or remove these pathogens effectively, resulting in a final product with a high margin of safety.

Aims: To confirm that the pasteurization step of CSL Behring's coagulation and wound healing therapies (antihemophilic and von Willebrand factors, prothrombin complex, factor XIII, factor IX, fibrinogen, and thrombin) is effective for inactivating emerging viruses that may be present in the donor population.

Methods: Process intermediates were spiked with emerging (or appropriate closely related model) viruses and pasteurized (subjected to heat treatment in aqueous stabilized solution at 60 °C for 10 h). Samples were removed at intermediate time points and evaluated for residual infectivity.

Results: Model viruses for SARS-CoV and MERS-CoV (SARS-CoV and transmissible gastroenteritis coronavirus), CHIKV (Sindbis and Semliki Forest viruses), and DENV (bovine viral diarrhea, yellow fever, and West Nile viruses) were completely inactivated by pasteurization, resulting in high virus reduction factors. HEV was completely inactivated by the pasteurization step (assessed using a piglet infectivity model).

Conclusion: These studies provide confirmation that pasteurization is a highly effective virus inactivation step for a wide variety of enveloped

and non-enveloped emerging viruses and significantly contributes to the safety of the final product(s).

Disclosure of Interest: N. J. Roth Employee of: CSL Behring, W. Schäfer Employee of: CSL Behring, T. Nowak Employee of: CSL Behring, B. Popp Employee of: CSL Behring, J. Schlosser Grant/Research Support from: CSL Behring, M. Eiden Grant/Research Support from: CSL Behring, M. Groschup Grant/Research Support from: CSL Behring, A. Gröner Employee of: CSL Behring.

PO606-WED

Platelets are dispensable during the initial steps of immunological transfusion related acute lung injury (trali)

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Background: TRALI is a severe accident of transfusion, defined as a non-cardiogenic pulmonary edema occurring within 6 h after blood transfusion. The exact role of platelets in TRALI remains controversial.

Aims: To evaluate the role of blood platelets in an experimental 2-hit mouse model of TRALI.

Methods: TRALI was induced in H-2d⁺ mice primed with LPS 24 h before challenge with an anti-MHCI monoclonal Ab (mAb). The role of platelets was investigated by i) inducing thrombocytopenia in transgenic iDTR mice selectively expressing diphtheria toxin (DT) receptor in megakaryocytes ii) administering a rat anti-GPIIb/IIIa mAb in BALB/c mice iii) inhibiting their reactivity with aspirin or clopidogrel or iv) transfusing platelets deficient for the α_{IIb} integrin into severely thrombocytopenic H-2d⁺ mice.

Results: 10 min after anti-MHCI mAb injection, TRALI reactions, namely lung edema and fall of aortic blood oxygenation, were similar in severely thrombocytopenic DT-iDTR mice and in control mice, without signs of lung hemorrhage. At the 2 h time-point, mortality was exacerbated in DT-iDTR mice due to alveolar hemorrhage, resulting in confounding effect about the actual role of platelets in the development of TRALI. When a less severe thrombocytopenia was induced using an anti-GPIIb/IIIa mAb, TRALI was not hemorrhagic and its development was similar to that in control mice. TRALI reactions developed similarly in mice treated with aspirin or clopidogrel, or transfused with α_{IIb} -deficient platelets. Finally, TRALI-associated early events were abrogated after depletion of neutrophils or administration of scavenging reactive oxygen species (ROS) in thrombocytopenic mice.

Conclusion: Initiation of TRALI is driven by neutrophils and the generation of ROS, while platelets are dispensable. Although a role of platelets on TRALI-associated pathologic events cannot be excluded in later steps, inhibition of specific activation pathways is unable to impact their development.

Disclosure of Interest: None declared.

PO607-WED

Volume of fluid resuscitation in women with persistent postpartum haemorrhage and total blood loss, maternal mortality and severe maternal morbidity

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Background: Large volumes of crystalloids and colloids might aggravate bleeding by causing dilutional coagulopathy in patients with major blood loss. Therefore, it is essential to investigate the clinical relevance of this effect within the range of fluid volumes administered to women with persistent postpartum haemorrhage (PPH) in current practice.

Aims: To assess whether large volumes of crystalloids and colloids lead to more blood loss, maternal mortality and severe maternal morbidity in women with persistent PPH, compared to infusions of small volumes of fluids.

Methods: From a cohort of women that in 2011–2013 received either ≥ 4 units of red blood cells or a multicomponent transfusion within 24 h following delivery, we selected women with persistent PPH (≥ 1000 mL), defined as PPH refractory to first-line uterotonic and surgical therapy, depending on the cause of PPH. Outcomes were total blood loss, maternal mortality and severe maternal morbidity. Patients were categorized into quartiles of the total fluid volume received prior to red blood cells transfusion, with the lowest volume category as reference.

Results: Median blood loss for the 880 included women was 3.0 L (interquartile range 2.5–4.0), and 33.3% of included patients experienced adverse outcomes. Women in the highest quartile of “fluid received prior to red blood cell transfusion” ended up (1) losing much more blood than women in the lowest quartile - adjusted regression coefficient for the highest volume category (≥ 4.3 L) was 1.3 (95% confidence interval 1.0–1.7), and (2) having many more adverse maternal outcomes, adjusted OR 3.6 (95% CI 2.3–5.7). For the second and third quartiles results were similar to the reference group.

Conclusion: Large fluid volumes before blood transfusion were associated with statistically significant more total blood loss and worse maternal outcomes.

Disclosure of Interest: None declared.

PO608-WED

Rapid assessment of platelet function under flow following trauma & recapitulation of trauma-induced coagulopathy in whole blood microfluidic assays

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Background: Trauma-induced coagulopathy is multifactorial and platelet function following trauma is not well characterized. Microfluidic assays can recreate the hemodynamic conditions required to assess platelet function.

Aims: To characterize the pathways underlying platelet dysfunction in trauma patients and to recapitulate hematocrit (Hct) dilution and hyperfibrinolysis under flow with modified healthy blood.

Methods: We assessed platelet activation (flow cytometry) and function (microfluidic assays) in trauma patients from the Level 1 Trauma center at the Hospital of University of Pennsylvania. Microfluidic

assays were also used to examine effective hematocrit (Hct; 10%, 20%, 30%) dilution by saline, platelet poor plasma, or platelet rich plasma. Exogenous addition of tPA (0–50 nM) promoted hyperfibrinolysis under flow. Platelet and fibrin deposition was measured with corn trypsin inhibitor (CTI; FXIIa-inhibited) or PPACK-inhibited whole blood perfusion over tissue factor bearing collagen type I surfaces or collagen type I surfaces.

Results: PPACK-inhibited whole blood testing from trauma patients under flow showed deficits in platelet GPVI signaling and secondary platelet aggregation in 9 of 20 patients. Flow cytometry showed a decrease in p-selectin expression upon stimulation with ADP or ADP and collagen of trauma platelets. Hct dilutions in healthy blood resulted in delayed platelet adhesion and decreased total platelet accumulation by 21%, 26%, and 77% respectively ($n = 40$ clots). Effective hematocrit dilutions also decreased total fibrin accumulation at 10% and 20% Ht (41% and 57% respectively). Activation of the lytic state with tPA resulted in a 91% reduction in total fibrin accumulation ($n = 20$ clots) and was detectable in < 15 min.

Conclusion: Microfluidic assays effectively assessed platelet dysfunction in trauma patients. This platelet dysfunction seen in trauma patients under flow indicates a potential rapid 15 min diagnostic strategy to help guide coagulopathy treatments.

Disclosure of Interest: None declared.

PO609-WED

Monitoring platelet transfusion in thrombocytopenic patients with hematologic malignancies measured by thromboelastometry: a pilot study

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Background: Prophylactic platelet transfusion is often given at a total platelet count of $< 10 \times 10^9/L$ in hemato-oncological patients. However, platelet count alone is a poor predictor of bleeding. In search of alternative predictors and protectors of bleeding, thromboelastometric (ROTEM[®]) evaluation of platelet transfusion was performed.

Aims: Assessing the effect of platelet transfusion on ROTEM[®] parameters in thrombocytopenic hemato-oncological patients.

Methods: Blood samples from adult hemato-oncologic patients, receiving prophylactic platelet transfusion on the Maastricht University Medical Centre+ hematology ward, were analyzed pre- and posttransfusion (1 and 24 h) using standard coagulation tests (aPTT, PT, fibrinogen, platelet count) and ROTEM[®] (EXTEM, FIBTEM) measurements. Platelet component was calculated.

Results: A total of 4 patients receiving 5 platelet transfusions were included. The corrected count increment was 11.2 and 7.1 1 h and 24 h after transfusion. Fibrinogen was unchanged after transfusion. The EXTEM maximum clot firmness (MCF) significantly increased 1 h after platelet transfusion (30 mm vs. 45 mm, $P = 0.043$). The FIBTEM MCF was not different before and after transfusion. The EXTEM clot formation time (CFT) was significantly shorter 1 h after transfusion than before (562 sec vs. 210 sec. $P = 0.043$). The platelet component increases significantly 1 h after transfusion (17.9 vs. 61.3 $P = 0.04$). There was a positive correlation between de TPC and MCF in the EXTEM ($r_s = 0.97$, 95%CI 0.91–0.99).

Conclusion: These results need to be interpreted with care, because of the small patient numbers. One hour after platelet transfusion, clot firmness, the speed at which the clot is formed and the platelet component increase significantly. There is a positive correlation between TPC and the EXTEM MCF. This, and the lack of significant differences in FIBTEM measurements indicate that the observed increases are mainly attributable to platelets.

Disclosure of Interest: None declared.

PO610-WED

Characterization and evaluation of the hemostatic action of cryopreserved platelets

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Background: Cryopreserved platelet concentrates (CRYO-PLT) are the best alternative for severe cases of alloimmunization and incompatibilities in thrombocytopenic patients who require urgent transfusion.

Aims: Characterize the impact of the cryopreservation process on different indicators of platelet integrity and in their hemostatic capacity.

Methods: Presence of major glycoproteins and expression of activation markers were evaluated by flow cytometry. We investigated the ability of CRYO-PLT to generate thrombin in response to tissue factor, and assessed their hemostatic capacity in flow devices using thrombocytopenic blood circulated through chambers exposing a damaged vessel.

Results: Cryopreservation decreased platelet counts and increased the presence of microparticles. Presence of constitutive glycoproteins (GPIIb/IIIa, GPIb and GPIV) was reduced. Expression of platelet activation markers (CD62-P and CD63) and procoagulant antigens (FVa and anionic phospholipids) was increased. Kinetics of thrombin generation revealed acceleration of dynamic parameters, with an elevation of thrombin peak with CRYO-PLT (542.3 ± 36.2 nM vs. 403.0 ± 24.4 nM with fresh platelets; $P < 0.01$). Perfusion studies demonstrated a reduction in the interaction of platelets with the vessel surface with a significantly enhanced presence of fibrin deposited on damaged vascular surfaces.

Conclusion: The cryopreservation procedure has detrimental effects on platelet glycoproteins, increases procoagulant markers, potentiating dynamics of thrombin generation. Studies under flow conditions confirm impairment in the adhesive and aggregating capacities of CRYO-PLT, with an increased procoagulant potential that could compensate their ability to restore hemostasis after transfusion.

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PO611-WED

Thrombin generation potential of a new trehalose-stabilized platelet-derived hemostatic agent

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Background: Stabilization of individual cells or entire organisms with trehalose permits retention of essential structure and functions through the stresses of dehydration and reconstitution. Cellphire Inc has developed a proprietary process to apply this principle to produce a potentially infusible freeze-dried platelet-derived hemostatic agent (PDHA) called Thrombosomes[®]. Characterization of the potency of this product for transfusion relies on several measures of function and responsiveness; the thrombin generation assay (TGA) data from two different systems are presented herein.

Aims: To compare the thrombin generation potential of Thrombosomes[®] under TGA conditions of high or low tissue factor (TF) and low phospholipids (PL) to see the contribution of catalytic surface activity vs. coagulation initiation.

Methods: Thrombosomes[®] were assayed in the Technoclone TGA with reagent A (low PL, no TF) and the Thrombinoscope TGA with PRP reagent (low PL, with TF). Dilutions were made in the presence of pooled normal plasma filtered to remove microparticles.

Results: The lag times were much longer in the Technoclone assay (no TF) but the total thrombin formed was much higher. Also in this assay, the Thrombin Peak height measured as background was at 75–80% of the levels achieved at the highest Thrombosomes[®] concentration vs. only 40% in the Thrombinoscope (with TF). In both assays the parameters most responsive to the dose of Thrombosomes[®] were the Peak height and the time to Peak. With the background values subtracted the dose-response curves for Thrombin Peak were similar in both assays; thus it suggests that Thrombosomes[®] present both PL and TF characteristics in thrombin generation. The Thrombin Peak parameter was found to be a sensitive indicator of changes in Thrombosomes[®] induced by vortexing or multiple centrifugations and storage.

Conclusion: Conclusions: These findings support the value of TGA as a quality control index for PDHA characterization in manufacture. Federal funds: Biomed Adv Res Dev Auth DHHS

Disclosure of Interest: A. Bode Employee of: Cellphire, N. Tandon Employee of: Cellphire, E. Matveeva Employee of: Cellphire, M. Fitzpatrick Employee of: Cellphire.

PO612-WED

Asphyxia by drowning induces massive bleeding due to hyperfibrinolytic disseminated intravascular coagulation

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Background: To date no study has systematically investigated the impact of drowning induced asphyxia on haemostasis.

Aims: Our objective was to test the hypothesis that asphyxia induces bleeding by hyperfibrinolytic disseminated intravascular coagulation (DIC).

Methods: All cases of drowning induced asphyxia ($n = 49$) were compared to other patients with cardiopulmonary resuscitation ($n = 116$) and to patients with acute promyelocytic leukemia (APL; $n = 83$). Six drowning victims were investigated prospectively. To study the mechanism, a forearm-ischemia model was used in 20 volunteers to investigate whether hypoxia releases tissue plasminogen activator (t-PA).

Results: Eighty percent of patients with drowning induced asphyxia developed overt DIC within 24 h. Compared to non-drowning cardiac arrest (CA) patients, drowning patients had a 13-fold higher prevalence of overt DIC on admission (55% vs. 4%; $P < 0.001$). Despite comparable DIC scores, APL patients had higher fibrinogen but lower D-dimer levels and platelet counts than drowning patients ($P < 0.001$). Drowning victims had a 3-fold longer activated partial thromboplastin time (APTT, 124s; $P < 0.001$) than both non-drowning cardiac arrest and APL patients. Hyperfibrinolysis induced bleeding was reflected by up to 1000-fold increased D-dimer levels, > 100-fold elevated plasmin-antiplasmin levels and a complete absence of thrombelastometric clotting patterns which was reversed by antifibrinolytics and heparinase. Thirty minutes of forearm-ischemia increased t-PA 31-fold ($P < 0.001$).

Conclusion: The vast majority of drowning patients develops overt hyperfibrinolytic DIC, partly caused by hypoxia induced t-PA release. Antifibrinolytics and heparinase partially reverse the abnormal clot-

ting patterns. Severe APTT prolongation may be a marker of combined hyperfibrinolytic afibrinogenemia and auto-heparinization in drowning related asphyxia.

Disclosure of Interest: None declared.

PO613-WED

The stability of FVIII:C, von willebrand factor and fibrinogen in FFP and cryoprecipitate in thai blood donors

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Background: Fresh frozen plasma (FFP) and cryoprecipitate are the blood components of choice in the management of either inherited or acquired bleeding disorders. Low amount of FVIII:C and vWF in FFP and cryoprecipitate may be a result from O blood group, the processes of blood component preparation, etc. If a large volume of replacement is needed, ABO compatible cryoprecipitate may be advisable.

Aims: To demonstrate the normal level of FVIII:C and vWF of individual blood group of Thai blood donors and to measure the amount of FVIII:C, vWF and fibrinogen in FFP and cryoprecipitate at day 0 and day 90 after preparation.

Methods: During September to October 2014, after consented, healthy blood donors were assessed for bleeding score. FFP and cryoprecipitate were prepared as a standard protocol and were kept frozen at -20°C for 90 days. FVIII:C, a panel of tests of vWF and fibrinogen of citrated-plasma of donors, FFP and cryoprecipitate at day 0 and day 90 were measured.

Results: Out of 140 donors, 81 were men. The median age was 37 years old (range 18 to 58). The mean value of FVIII:C of O, B, A and AB blood group donors were 104, 137, 138 and 146 IU dL⁻¹, respectively ($P < 0.0001$, O VS non-O). vWF:Ag, vWF:Rco, vWF:CB and vWF:FVIII:B of O blood group donors were 98, 76, 95 and 87 IU dL⁻¹, respectively. Except vWF:FVIII:B, the amount and other functions of vWF among non-O donors were significantly higher than O blood group donors. The mean value of FVIII:C, vWF:Ag, vWF:Rco, vWF:CB, vWF:FVIII:B, fibrinogen in FFP and cryoprecipitate at day 0 were 106, 141, 93, 117, 82 IU dL⁻¹, 326 mg dL⁻¹, and 84, 136, 83, 127, 107 IU bag⁻¹, 139 mg bag⁻¹, respectively. The values of vWF in FFP and cryoprecipitate at day 90 were trivially lower than those at day 0.

Conclusion: The levels of FVIII:C and vWF in donors with O blood group and component prepared from O blood group were lower than those in non-O donors. The FVIII:C, vWF and fibrinogen levels in FFP and cryoprecipitate were stable at day 90 after preparation.

Disclosure of Interest: None declared.

PO614-WED

Infusion of cryopreserved pooled platelets in patients with acute leukemia: preliminary results of a clinical trial (NCT02032134)

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Background: The current use of fresh platelets (PLT) represents a logistical burden in cases of imbalance between request and availability of blood components; cryopreserved platelets (CRY-PLT) should overcome this limit.

Aims: To determine the efficacy and safety of CRY-PLT from buffy coat in patients with hematological disorders needing platelets transfusion for bleeding or prophylaxis

Methods: Using a patented system able to avoid external manipulations after thawing, we have preliminarily evaluated *in vivo* the availability and function of buffy coat derived pooled platelets cryopreserved with DMSO. CRY-PLT *in vivo* assays met the currently accepted criteria of efficacy up to 9 months after storage. We report on the results of CRY-PLT transfusion in hematological patients requiring platelets for prophylaxis or treatment of bleeding. After transfusion, the corrected count of platelets was determined at 1 h and 24 h, respectively.

Results: From June 2014 to January 2015 five patients were enrolled (4 males and one female) with a mean age of 71.6 years (range:65–83). Four patients had acute leukemia and one had aplastic anemia. All patients received one unit (170–200 mL final volume) of CRY-PLT prophylactically for very low PLT count (mean basal $5 \times 10^9/L$, range:2–7), secondary to chemotherapy in 3 cases. Three patients were refractory to previous PLT transfusion from aphaeresis and their PLT count only barely increased also after CRY-PLT. One patient showed a good response to CRY-PLT with an increase in PLT count after 1 h, stable up to 24 h while one patient had only a slight increase in PLT count. CRY-PLT transfusion did not determine any adverse event. Patients did not bleed. In two refractory cases, further PLT transfusions at 48 and 72 h after CRY-PLT, were associated with an unexpected good response

Conclusion: CRY-PLT determined a good increase in PLT count in one case but they were effective in preventing bleeding up to 48 h after transfusion and safe in all cases

Disclosure of Interest: None declared.

PO615-WED

Massive haemorrhage management: from chaos to order. legnano hospital's experience

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Background: Management of massive haemorrhage (MH), regardless of its aetiology, needs an active collaboration among the involved clinical team and the Transfusion and Haematology laboratory services. Before November 2012 our behaviour in this dramatic clinical occurrence was objectively chaotic and uncoordinated. Hemocomponents and hemoderivatives were released and transfused in a not standardized amount and were only seldom targeted to lab test results.

Aims: Starting from the overexposed picture, in December 2012 we undertook a multidisciplinary and multistep approach to MH.

Methods: After a revision of the recent literature and several meetings and discussions with all the involved professionals, we developed a shared MH protocol. We started the production of pooled cryoprecipitate, always subjected to quality control for fibrinogen (FBG) content. Whenever possible, haemotherapy was guided by viscoelastic test (ROTEM[®]) results. After implementation, all the more critical cases were audited in plenary meetings and any procedural flaw was evidenced and discussed. MH was formalised by the Hospital's Management in November 2013

Results: From 11/2013 to 06/2014 we managed 18 post-traumatic MHs. All patients (pts) were transfused with RBC (mean units 9. min 4 – max 17) and plasma (mean 1700 mL. min 795 – max 3165). 66% percent of pts underwent PP transfusions (mean units 1.75. min 1 – max 4). The mean RBC/PFC ratio was 1.4. Two thirds of pts had a fibrinogen concentration below the established target (150 mg mL^{-1}) and were transfused with cryoprecipitate (mean FBG administered/transfusion episode 3.2 gr). Seventeen pts out of eighteen survived (94.5%). Retrospective data analysis showed an inappropriate MH protocol activation in one case (5.5%). As time went by, the transfusionist became a trusted consultant rather than an automatic blood dispenser.

Conclusion: The implementation of a MH protocol helped us to provide a faster and more appropriate treatment of this life-threatening condition.

Disclosure of Interest: None declared.

PO616-WED

Interventions to reduce blood loss from laboratory testing in critically ill patients and impact on transfusion: a systematic review

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Background: Blood loss from laboratory testing contributes to anemia, which is a common complication of critical illness and is associated with poor outcomes. Reduction of diagnostic phlebotomy volume may reduce transfusions and improve clinical outcomes in critically ill patients.

Aims: To identify strategies for reducing blood loss from diagnostic phlebotomy and assess their impact on volume of blood loss, transfusion and clinical outcomes.

Methods: Systematic review of published English-language studies using standard methodology.

Results: A search (Medline and Embase) yielded 3045 studies. After review by 2 independent assessors, 7 studies including 3 randomized controlled trials and 4 controlled cohort studies were included. Interventions included indwelling catheter devices that reduce discarded blood ($n = 4$), small-volume blood collection tubes ($n = 2$) and avoidance of arterial catheters ($n = 1$). All 3 RCTs reported reduced daily phlebotomy volumes using arterial catheter blood conservation devices compared to standard practice (mean/day 35 vs 69 mL, $P < 0.01$; median/day 8 vs 40 mL, $P < 0.001$; median/day 63 vs 133 mL, $P = 0.001$). Two RCTs reported transfusion data, 1 of which showed a statistically significant decrease in transfusions with the arterial catheter device intervention vs. control (21% vs. 38%, $P = 0.01$). In 2 of 3 cohort studies interventions reduced phlebotomy volumes compared to standard practice (mean/day 63.6 vs 114.7 mL, $P < 0.001$; median/day 5.1 vs 19.9 mL, $P < 0.001$; mean/day 32.2 vs 55.6 mL, p -value not specified, respectively). ICU length of stay was not different between groups.

Conclusion: Diagnostic phlebotomy volume reduction techniques appear to reduce blood loss and may reduce transfusion rates in critically ill patients. High methodological quality randomized studies evaluating patient-important clinical outcomes are needed to evaluate

the efficacy and possible harms associated with interventions that reduce blood loss for laboratory testing.

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PO617-WED

Comparison of the effect of fresh frozen plasma pre- and post-transfusion in ITU patients on standard and global coagulation assays

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Background: Fresh Frozen Plasma (FFP) is commonly used in the correction of acquired coagulopathy. Dose and response are routinely monitored by standard assays (PT, INR). Global assays are increasingly being used in algorithms to guide transfusion, although transfusion triggers and targets are not well defined.

Aims: To compare the response to FFP infusion using standard and global assays – PT, INR, ROTEM, thrombin generation (TGA).

Methods: Citrated whole blood samples (CWB) were collected from 16 patients on ITU pre- and post-FFP infusion (30 min, 2–4 h). Ethical approval was obtained from the UK National Research Ethics Committee. Informed consent was waived. PT/INR were tested as per routine laboratory practice. CWB were analysed using the NATEM test on the ROTEM. TGA was run as per Hemker *et al.* using 5 pM tissue factor and platelet poor plasma.

Results: 3/16 patients either did not have follow-up samples or results for all parameters. The INR was > 1.5 in 14/16 patients tested and only 3/16 corrected to < 1.5 . 15 patients were analysed by ROTEM (NATEM). 5/15 had prolonged baseline CT of which 2 normalised at 30 min and one patient normalised at 2 h. 3/14 had a prolonged tMax at baseline. One patient normalised at 30 min and a further patient at 2 h. ROTEM angle parameter was decreased in 3/15 patients of which 1 had corrected at both time points. 4/15 had decreased MCF of which 1 patient corrected at 30 min and 2 were within normal range at 2 h. of those with an abnormal CFT and Max V (4/15; 3/14) no correction was seen. ETP measured by TGA was suppressed in 5/15 and remained so despite FFP.

Conclusion: The PT/INR does not show significant response to FFP. Approximately 1/3 of patients had abnormal baseline ROTEM (NATEM) and TGA parameters of which a minority in the former showed correction into the normal range post transfusion. This com-

parison brings into question the most appropriate trigger for FFP transfusion and underlines the problem of monitoring response by laboratory assays.

Disclosure of Interest: None declared.

Venous thromboembolism-diagnosis III

PO618-WED

Serum albumin and S protein are better predictors than clinical scores in the diagnosis of deep venous thrombosis in patients with several comorbidities

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Background: The panorama of Deep Venous Thrombosis (DVT) is being challenging in the last years, this is putting the Emergency Department context into Internal Medicine context in the matter of patients with chronic diseases hospitalized for several comorbidities. Furthermore, DVT in sites other than lower limbs is increasingly being recognized as a clinical entity. The relationship between score models, based on risk factors, symptoms and clinical signs of lower limbs DVT, and circulating biomarkers is not well studied.

Aims: Compare the different scores and the additional value of circulating biomarkers in the prediction of DVT in patients hospitalized in Internal Medicine Department.

Methods: We prospectively recruited 178 consecutive patients who presented a D-dimer value > 500 ng mL⁻¹. In each patient Wells modified score for DVT, Hamilton score, Kahn score and St. André Hospital score were applied. We measured serum levels of albumin (%) and protein S (%). Diagnosis of DVT was made by Extended Compression Ultra-Sonography (ECUS) standardized procedure. All patients underwent also an angiographic-CT-scan.

Results: In 85 (48%) patients the diagnosis of DVT was confirmed. Sites of DVT were as follows: Lower limbs venous thrombosis $n = 71$ (84%); Upper limb venous thrombosis $n = 8$ (9%); portal vein thrombosis $n = 6$ (7%). Patients with venous thrombosis had significantly lower albumin levels as compared with controls (mean $\pm$ SD 47.6 ± 7.9 vs $55.4 \pm 4.6\%$, respectively, $P < 0.001$) and lower Protein S (60.7 ± 26.3 vs. $87.6 \pm 11.9\%$, $P < 0.001$). The best predictor is protein S with a very high PPV (93%) and a good accuracy (AUC 0.821). In this setting, the use of clinical risk scores based on symptoms and clinical signs showed low accuracy (AUC 0.690), low PPV (59%) and satisfactory NPV (74%).

Conclusion: this study demonstrates for the first time that serum albumin and protein S have a better accuracy than clinical scores in the diagnosis of DVT in hospitalized patients.

Disclosure of Interest: None declared.

PO619-WED

Derivation of a new score to predict chronic thromboembolic pulmonary hypertension after acute pulmonary embolism

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Background: Validated risk factors for chronic thromboembolic pulmonary hypertension (CTEPH) after acute pulmonary embolism (PE) are currently unknown.

Aims: The aim of the present study was to construct a clinical score to predict CTEPH development after acute PE based on simple and reproducible patient characteristics assessed at the moment of PE diagnosis.

Methods: We combined 3 observational cohorts of consecutive PE patients who underwent echocardiography after a median of 1.5 years, if abnormal followed by additional diagnostic tests to confirm CTEPH. Baseline demographics and clinical characteristics were included in a multivariate regression analysis. Independent predictors were combined in a risk stratification score.

Results: of 772 patients with complete follow-up, CTEPH was ruled out in 711 (92%) and confirmed in 22 (2.8%) by right heart catheterization (RHC). CTEPH was 'probable' although RHC was not performed in 12 and 'unlikely' but not completely ruled out in 27 patients. The former patients with 'probable' CTEPH were included in the sensitivity analysis, the latter excluded from further analysis. Unprovoked PE (+6 points), hypothyroidism (+3 points), symptom onset > 2 weeks before PE diagnosis (+3 points), right ventricular dysfunction on CT or echocardiography (+2 points), diabetes mellitus (-3 points), and thrombolytic therapy or embolectomy (-3 points) independently predicted CTEPH. The area under the curve (AUC) of ROC curve of the clinical score including those 6 variables was 0.89 (95%CI 0.84–0.94). Sensitivity analysis and bootstrap internal validation confirmed this AUC. Seventy-three percent of patients were categorized to low risk category (CTEPH incidence 0.38%, 95%CI 0–1.5%) and 27% to high risk category (CTEPH incidence 10%, 95%CI 6.5–15%).

Conclusion: The proposed score is based on previously identified and new clinical variables associated with CTEPH, all assessed at moment of PE diagnosis. If externally validated, the score may guide targeting CTEPH screening to high risk patients.

Disclosure of Interest: None declared.

PO620-WED

The prevalence of deep vein thrombosis in hospitalized patients with suspected pulmonary embolism ruled out by multislice CT angiography

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Background: Although multislice CT angiography (CTPA) can safely rule out pulmonary embolism (PE) without the need of additional leg ultrasonography (CUS) in outpatients, in a preliminary study we

recently observed that in hospitalized patients with suspected PE ruled out by CTPA the prevalence of deep vein thrombosis (DVT) was increased up to six percent.

Aims: To provide a more accurate estimate of the prevalence DVT in hospitalized patients with suspected PE ruled out by CTPA.

Methods: Prospective single center cohort study. Patients were included if they had developed symptoms compatible with PE more than 48 h after being admitted to hospital for any reason other than PE or more than > 24 h after being admitted for a major surgical intervention (general anesthesia time > 90 min). All participants were evaluated with CTPA (64-slice or greater scanner). Patients were excluded if they had symptoms compatible with PE prior to admission. After providing written consent, all patients were evaluated with bilateral whole leg CUS.

Results: Between November 2011 to December 2014, 176 hospitalized patients were included (average 1.1 patients per week). Forty three percent had active cancer, 58% had major surgery and 77.3% were on thrombo-prophylaxis). Shortness of breath was the presenting symptom in 73.3% of the patients, 22.2% had symptoms of DVT and 48.2% had a Wells score > 4 points. The overall prevalence of PE was 35.8% (95% CI 28.7–43.4), and 11% (95% CI 4.5–21.5) in those with a Wells score ≤ 4. The prevalence of proximal DVT was 15.9% (95% CI 7.8–27.2) in those with PE vs. 7.07% (95% CI 3.1–13.4) in those without PE; the incidence of distal DVT 20.6% in those with PE vs. 5.4% in those without PE. In those without PE, the presence of symptoms of DVT increased the risk proximal DVT (RR 5.9; 95% CI 3.2–10.7).

Conclusion: Given the high prevalence of proximal DVT in hospitalized patients in whom PE is ruled out by a negative CTPA, a proximal bilateral CUS could be required to safely exclude PE.

Disclosure of Interest: None declared.

PO621-WED

Cerebral and splanchnic vein thrombosis: what molecular genetics risk factors?

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Background: Cerebral and splanchnic venous thrombosis are rare events. Molecular genetics abnormalities differ between deep venous thrombosis and those two types of venous thrombosis. For example, factor V Leiden mutation is more frequent in deep venous thrombosis while prothrombin g20210a mutation is more frequent in cerebral and splanchnic vein thrombosis.

Aims: The aim of this work was to study several polymorphisms in coagulation factors (II, V, XIII) or coagulation inhibitors (Protein C, Protein Z) able to influence risk of venous thrombosis in 118 cerebral venous thrombosis (CVT), 53 splanchnic thrombosis (ST) and 101 healthy controls. Moreover, we studied prevalence of *JAK2* V617F mutation and Calreticulin (*CALR*) exon 9 mutations in CVT and ST.

Methods: All patients and healthy controls gave informed consent for genetic analysis. In the hemostasis unit, all polymorphisms were studied by PCR and enzymatic restriction. *JAK2* V617F mutation was studied by allele specific PCR and mutations of exon 9 of *CALR* were studied by PCR followed by Sanger's sequencing.

Results: Firstly, we confirmed the important role of prothrombin g20210a mutation in CVT and ST with an odds ratio respectively of 17.9 and 11.9 compared to the healthy controls. Moreover the g79a polymorphism in protein Z gene influenced the risk of CVT but not of ST. The prevalence of *JAK2* mutation was 4.3% and 37.2% respectively in CVT and ST, with or without myeloproliferative disease. Finally, no patient carried a mutation in *CALR* exon 9 gene.

Conclusion: Some coagulation polymorphisms are risk factors of CVT or ST. In contrast, *JAK2* mutation was very more frequent in ST than in CVT and has to be assayed in this disease. *CALR* mutation is not a risk factor of CVT and ST.

Disclosure of Interest: None declared.

PO622-WED

A prospective evaluation of venous thromboembolism in spine surgery: incidence, cost-effectiveness of prophylaxis and bleeding complications

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Background: Limited data are available on the incidence of VTE, efficacy and cost-effectiveness of prophylaxis in patients undergoing elective spine surgery.

Aims: To assess the VTE incidence, cost-effectiveness of low molecular weight heparin (LMWH) compared to unfractionated heparin (UH) and associated bleeding complications.

Methods: Lower extremity venous duplex scans were performed in 369 consecutive patients (1 week pre- and 4–6 weeks post-operatively). Demographics, diagnosis, hospital length of stay (LOS), VTE risk factors, prophylactic modalities, operative details and bleeding risk were analyzed. An economic model to simulate the impact of UH or LMWH on the incidence and treatment costs for deep vein thrombosis (DVT), pulmonary embolism (PE), heparin induced thrombocytopenia (HIT), post-thrombotic syndrome (PTS) and bleeding was created from local treatment algorithms and randomized trials. Costs are expressed in Australian Dollars (AUD).

Results: Median age was 58 years (48–67), LOS was 7 days (4–10) and operative duration was 2.4 h (1.6–3.5). LMWH was used in 21.7%, UH in 24.7%, UH followed by LMWH in 1.6% and no pharmacological prophylaxis in 52% of patients. All received intermittent pneumatic compression. VTE incidence was 3.5%, 3.3% for DVT (50% post discharge) and 0.8% non-fatal PE. An increase in LMWH use reduced annual inpatient hospital costs from \$287,504 to \$255,831. Major bleeding increased for every hour of surgery (OR: 2.5; 95% CI: 1.5–4.2; $P = 0.01$) as did expenditure (by \$22,379). Estimated annual cost savings with LMWH was \$33,615 for DVT, \$7,640 for PE, \$18,503 for PTS and there were fewer bed days.

Conclusion: Our in-hospital VTE was relatively low. Bleeding risk was influenced by operative duration. A combination of pharmacological and mechanical prophylaxis is beneficial in patients with multiple risk factors however DVT risk persists beyond discharge. Savings are reflected by a reduction in costs for treatment of major bleeding, VTE, PTS and fewer hospital bed days.

Disclosure of Interest: None declared.

PO623-WED

Thromboembolic disease following pancreaticoduodenectomy for benign or confirmed malignancy: efficacy of prophylactic measures and associated bleeding complications

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Background: Pancreatic cancer is associated with the highest thrombotic rates (17% to 57%) amongst any other gastrointestinal cancers.

Aims: To assess the incidence of thrombotic events, efficacy of thromboprophylaxis and associated bleeding complications.

Methods: In total, 182 patients were retrospectively reviewed (2002–2014) that underwent pancreaticoduodenectomy for benign or confirmed malignancy. Demographics, diagnosis, venous thromboembolism (VTE) risk, hospital length of stay (LOS), operative duration, prophylactic modalities used and bleeding complications were assessed. In-hospital and 1 year readmission rate of all thromboembolic events were evaluated. This included portal vein thrombosis (PVT), upper-extremity deep vein thrombosis (UEDVT), lower extremity deep vein thrombosis (LEDVT) as well as pulmonary embolism (PE).

Results: Male to female ratio was 1.4: 1 with a median age of 63 years (55–71) and LOS of 20 days (12–31). Median operative duration was 4.5 h (3.8–6.3). Tumour localisation was mainly in the head of pancreas (39.6%) and ampulla of Vater (15.4%). Unfractionated heparin (UH) was used in 84.6% of patients, 7.7% received low molecular weight heparin (LMWH) and 7.7% used UH followed by LMWH. All received mechanical prophylaxis. Major bleeding occurred in 22.7% of patients on UH. In-hospital incidence for thrombosis was 7.1%, 2.2% for PVT, 2.7% for UEDVT, 1.1% for LEDVT and 1.1% for non fatal PE. The 1 year thrombotic incidence was 9.9% with 27.8% of late VTE events detected post hospitalization. VTE accounted for 5.4% of all readmissions. 1 year mortality was 23.1%.

Conclusion: The rate of in-hospital thrombotic events was relatively low highlighting the importance of optimal in-hospital thromboprophylaxis relative to risk factors, tumour grade, chemotherapy and/or radiotherapy, operative duration, LOS and bleeding risk. The out of hospital increase in thrombotic events suggests that high risk patients may possibly benefit from post-discharge thromboprophylaxis.

Disclosure of Interest: None declared.

PO624-WED

Performance of a diagnostic strategy using a clinical decision rule and D-dimer testing to exclude upper extremity deep vein thrombosis in clinically important subgroups

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Background: The diagnosis of upper extremity deep vein thrombosis (UEDVT) is challenging. A recent study demonstrated that an algorithm consisting of a clinical decision rule (CDR), D-dimer, and ultrasonography (US) can safely exclude UEDVT (Kleinjan et al., *Ann Intern Med*, 2014).

Aims: To evaluate the performance of this strategy in clinically important subgroups and to explore the potential of an age-adjusted D-dimer threshold.

Methods: Data on 406 patients with suspected UEDVT enrolled in an international diagnostic management study were used. We calculated the proportion of patients in whom US could be withheld based on the combination of an unlikely CDR and a normal D-dimer overall and in prespecified subgroups of patients with a central venous catheter (CVC) or pacemaker, cancer, age ≥ 75 years, and inpatients. The

changes when applying an age-adjusted D-dimer threshold (age in years $\times 10 \text{ ng mL}^{-1}$ in patients older than 50 years) were calculated.

Results: In 87 of 406 patients in the total study group (21%), an unlikely clinical probability score combined with a normal D-dimer test result excluded UEDVT, as reported before. None of these patients developed UEDVT during 3 months follow-up. In contrast, US could be withheld in only 4 of 92 patients with a CVC or pacemaker (4.3%; 95% CI 1.7% to 10.7%) and in 4 of 83 inpatients (4.8%; 95% CI 1.9% to 11.7%), compared to 10.9% in cancer patients and 12.5% in patients aged 75 years or older. Applying the age-adjusted D-dimer resulted in an additional 22 patients in whom US could have been safely withheld, increasing the corresponding efficiency from 21% to 27% (gain 5.4%, 95% CI 3.6–8.1%).

Conclusion: Although the combination of a clinical decision rule and D-dimer testing is safe in excluding UEDVT, its performance is limited in some clinical subgroups, in particular in inpatients and in patients with a CVC or pacemaker. Applying an age-adjusted D-dimer threshold could safely increase the performance of the diagnostic algorithm.

Disclosure of Interest: None declared.

PO625-WED

Prediction of venous thrombosis risk after arthroscopy of the knee

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Background: Orthopedic surgery is associated with a high risk of venous thrombosis (VT). In knee arthroscopy, reported VT incidence rates vary widely between 0.9% and 4.6% for symptomatic VT and between 3.1% and 17.9% for asymptomatic events. It is uncertain whether the benefits of thromboprophylaxis therapy outweigh its bleeding risk, and therefore guidelines are conflicting.

Aims: To explore the predictive values of environmental and genetic risk factors, levels of coagulation markers and other biomarkers on the occurrence of VT in patients who underwent arthroscopy of the knee. 2. To develop a clinical prediction rule to guide thromboprophylaxis decisions in these patients.

Methods: Data from a large population based case-control study (MEGA study, 4943 cases, 6294 controls) were used. Putative predictors were selected using univariate analyses (odds ratio > 1.2 $P \leq 0.05$). Predictors were included in our model using a forward stepwise selection method. To prevent overfitting, the multivariate analysis was performed in the whole study population while the Area Under the Curve (AUC) was only estimated in patients with arthroscopy. Apart from a 'full model' taking into account all variables, we also created a model with environmental factors only. Currently these models are being validated in 'THE VTE' study (case-control into aetiology on VT, 784 cases, 523 controls).

Results: Eight predictors (5 environmental, 2 coagulation factors, 1 genetic) were identified for our prediction model which resulted in an AUC of 0.84 (95% CI: 0.76–0.91). The model with environmental risk factors only resulted in an AUC of 0.73 (95% CI: 0.63–0.83).

Conclusion: We developed a prediction model for the development of VT in patients undergoing arthroscopy of the knee. Determination of at least 1 biomarker, in addition to only environmental factors, leads to accurate prediction of VT risk in these patients. This model can help clinicians to decide whether or not to prescribe thromboprophylaxis.

Disclosure of Interest: None declared.

PO627-WED

Relationship of patient affect to clinician-derived pretest probability assessment for pulmonary embolism

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Background: Previously, we showed that patients with PE manifest less positive emotional affect. Here we examine the association between clinician perception of positive affect (patient smile) and PTP assessment.

Aims: We hypothesize that patient's facial affect contributes to clinician expert intuition, which then informs the subjective components of pretest probability (PTP) assessment for pulmonary embolism (PE).

Methods: Prospective two center ED study of diagnostic accuracy. All patients had suspected PE and underwent computerized tomographic pulmonary angiography (CTPA) and 45 day follow up. Prior to knowledge of CTPA result, clinicians completed a standardized form that collected gestalt probability of PE on a visual analogue scale (0–100%), data for Well's score, and the question "Did the patient smile during your examination?" Criterion standard for PE was image-proven PE on CTPA.

Results: We enrolled 123 patients, 20 (16%) PE+. The mean gestalt PTP for PE+ was $41 \pm 23\%$ vs. $29 \pm 22\%$ for PE- patients ($P = 0.019$). Eleven PE+ patients smiled vs. 38 PE- (55% sensitivity and 14% specificity). For smile+ patients, the mean gestalt PTP for PE+ patients was $36 \pm 16\%$ vs. $26 \pm 21\%$ for PE- ($n = 38$) patients. For smile- patients, the mean gestalt PTP for PE+ was $46 \pm 19\%$ vs. $32 \pm 21\%$ for PE- patients. Comparing mean gestalt PTP for all smile+ ($27 \pm 20\%$) to all smile- ($34 \pm 21\%$) yielded $P = 0.04$ (t-test). The mean Wells score for PE+ was 5.1 ± 3.1 vs. 2.5 ± 2.4 for PE-. For smile+, mean Wells for PE+ was 3.5 ± 3.0 vs. 2.1 ± 2.2 for PE-. For smile-, mean Wells for PE+ was 6.8 ± 2.3 vs. 2.9 ± 2.5 for PE-. Comparing mean Well's score for all smile+ (2.4 ± 2.4) to all smile- (3.4 ± 2.8) yielded $P = 0.03$. Using Liddell's exact test, clinicians were 2.5 (95% CI 1.3–5.1) times more likely to categorize smile+ patients as having an alternative diagnosis more likely than PE.

Conclusion: These data suggest interdependency between physician perception of patients' emotional affect and clinical PTP assessment, which may influence decision-making.

Disclosure of Interest: None declared.

PO628-WED

Current practices in lung scintigraphy for pulmonary embolism (PE) diagnosis: an international survey in australia, canada and france

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Background: V/Q scintigraphy has been a rapidly evolving technology and many centres have switched from the conventional planar V/Q

scan interpreted with the PIOPED criteria to Single Photon Emission Computed Tomography (V/Q SPECT), sometimes combined with a low dose Computed Tomography (V/Q SPECT-IdCT)

Aims: To assess current practices in nuclear medicine centres in the context of acute PE suspicion

Methods: A survey including simple-choice questions was created online and sent to nuclear medicine centers in Australia, Canada and France from April to December 2014. The survey covered radiotracer, image acquisition, diagnostic criteria for SPECT and planar interpretation, and use of pseudoplanar images generated from SPECT data. Two sets of emails including an Internet link to the survey were initially sent. Departments were subsequently solicited by phone. A single response per department was consolidated

Results: 331 responses were collected (Australia: 74, Canada: 48 and France: 209). 72% of centers routinely use V/Q SPECT rather than planar imaging for acute PE suspicion. Routinely, V/Q SPECT is used in 34%, V/Q SPECT-IdCT in 31% and V/Q planar in 28% of centres with a strong variability among countries. The criteria recommended by the European Guidelines (binary interpretation, i.e. "PE" or "no PE", with a fixed diagnostic cut-off) are the most commonly used (60%) for SPECT reading. The criteria used for planar interpretation are very heterogeneous (EANM criteria: 35%, PIOPED: 29%, no standardized criteria: 31%)

Conclusion: Our study shows that a wide majority of nuclear medicine departments routinely uses V/Q SPECT rather than planar imaging in the context of acute PE suspicion, with inconsistent criteria for interpretation. This is a major message for PE diagnosis since no diagnostic strategy based on V/Q SPECT has been prospectively validated to date. This highlights the need for a better information to the clinician on the used technologies and the urgent need for a formal management outcome study

Disclosure of Interest: None declared.

Venous thromboembolism - epidemiology III

PO629-WED

Comparison of risk factors and outcomes associated with community (CAT) vs. hospital acquired thrombosis (HAT)

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Background: HAT has been subjected to scrutiny since 2005, in England. Limited data on CAT events, occurring twice as frequently as HAT but no official process for investigation.

Aims: To review all VTE events diagnosed in 2010 within Derriford Hospital, comparing risk factors for HAT and CAT and rates for recurrence and mortality over following 3 years

Methods: Over 3 years, positive VTE events identified using radiology data have been cross-checked with the patient management system to identify HAT events and all cause mortality. Notes review undertaken to determine risk factors. Five groups investigated: HAT deep vein thrombosis (DVT) and pulmonary embolism (PE), CAT DVT & PE and incidental findings (IFT - where unexpected VTE on scans mainly for cancer investigation). Parameters analysed using SPSS statistics package to identify significant associations.

Results: 604 VTE events identified in 2010. Of 406 CAT events, 223 were PE & 183 DVT. 198 HAT events comprised 116 PE and 82 DVT; statistically significant association of PE with HAT (p 0.003), with a crude incidence rate of 1.56 per 1000 patient years (95% CI 1.45–1.68). Age > 60 years significantly associated with HAT over CAT (p 0.002). Cancer was less associated with CAT DVT compared to other three cohorts (p 0.01). Family history, personal VTE history and obesity

had significantly higher associations with CAT than HAT (p 0.003, 0.001 & 0.001 respectively). Twelve-month mortality: CAT 55/421 13%, HAT 63/201 31% and IFT 49/66 74%. Three year mortality: CAT 83/421 20%, HAT 94/201 47% & IFT 59/66 89%; a significant association of mortality with HAT and IFT compared to all-cause mortality (p 0.001). 28 (25 DVT, 3PE) recurrences for all DVT patients and 23 recurrences, (19 PE, 4 DVT) for all PE patients.

Conclusion: Risk factors are significantly different for CAT vs. HAT events. Mortality associated with VTE is significantly greater for HAT and IFT compared to CAT events. Location of VTE recurrence is likely to be the same as the index event.

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PO630-WED

Association between venous thromboembolism (VTE) minor risk factors and the risk of recurrence after a first unprovoked episode

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Background: It has been shown that the risk of recurrence of Venous Thromboembolism (VTE) is significantly lower when provoked by a recent major risk factor such as surgery or traumatism, as opposed to an unprovoked VTE.

Aims: In this study we aimed at assessing the association between exposure to remote major risk factors and the risk of recurrence.

Methods: We performed a post-hoc analysis of data from the REVERSE cohort study, which enrolled patients with a first proximal deep vein thrombosis or PE not provoked by a major risk factor. This was defined as; VTE event in absence of fracture, plaster cast, immobilization greater than 3 days or general anaesthetic in the last 3 months prior to the VTE occurrence; a known deficiency antithrombin, protein C or S; and malignancy in the last 5 years. Patients were followed over 18 months after the completion of 5–7 months on OAT. We tested several remote risk factors including immobilization, surgery, lower extremity fracture or trauma between 3 months and 1 year prior to the diagnosis of VTE, or history of malignancy in the > 5 years (patients with active malignancy were excluded). The proportion of patients with recurrent VTE during follow up was determined according to the presence or absence of each of these risk factors.

Results: 646 patient were recruited from 2002–2006. The study included 332 males and 314 females with mean age of 55 years and BMI, Kg m⁻² of 29. 91 of 646 patients had an adjudicated VTE recurrence after a mean 18 months of follow-up. None of the remote major risk factors were found to be associated with a significantly lower risk of subsequent VTE during follow-up.

Conclusion: In patients after a first VTE that was not provoked by recent exposure to a major risk factor, remote major risk factors were not associated with the risk of recurrence at treatment discontinuation. Patients with exposure to one of these remote major risk factors with a first VTE should not be managed differently than patients with no VTE risk factors.

Disclosure of Interest: None declared.

PO631-WED

Do patients who experienced venous thromboembolism despite thromboprophylaxis during a medical immobilization have a worse outcome ?

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Background: Thromboprophylaxis is recommended for patients immobilized for acute medical diseases as chronic heart or respiratory failures, stroke, cancer or infection. It is unclear if patients who experienced venous thromboembolism (VTE) despite Thromboprophylaxis (T+ group) have a different outcome than those in whom Thromboprophylaxis was not prescribed (T- group).

Aims: Search for a poorer prognosis in patients who presented with VTE despite thromboprophylaxis.

Methods: We used the RIETE database to compare the outcome (death, fatal pulmonary embolism, VTE recurrences, major bleeding) during the first 3 months of all patients with objectively confirmed symptomatic VTE in the 2 months after an immobilization for an acute medical disease, according to the use of thromboprophylaxis.

Results: Thromboprophylaxis had been prescribed in 1313 (37.2%) of the 3527 included patients. Acute infection was the most frequent cause of immobilization (46% of T+ and 43% of T-, ns). Compared to T- patients, T+ patients were more frequently immobilized in hospital (70% vs 22%), but less frequently for cancer (13% vs 22%). VTE occurred during immobilization in a hospital in 1456 patients, 648 (44%) of them receiving thromboprophylaxis. VTE therapy was similar among the two groups. In univariate analysis, rates of death (23 vs 21%), fatal PE (2.6 vs 3.1%), VTE recurrences (2.4% vs 2.8%) and major bleeding (4.2% both) did not differ between T+ and T- groups, during the first 3-months of therapy. Even when adjusted to prognostic factors in multivariate models, Thromboprophylaxis was not associated with one of these outcomes.

Conclusion: We failed to find a different outcome in patients who presented with VTE despite thromboprophylaxis (prescribed during an acute medical disease) when compared to those for whom thromboprophylaxis was omitted. However, it remains that thromboprophylaxis is still underprescribed in medical patients, and several symptomatic VTE might be avoided with better thromboprophylaxis.

Disclosure of Interest: None declared.

PO632-WED

Discrepancies in severe RI screening in the elderly with cockcroft-gault and mdrd formulas: 20% of patients are misevaluated

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Background: For the past 10 years, international and inter-disciplinary harmonization for renal function estimation has been recommended. In 2005, the international KDIGO group published an official definition and classification of kidney disease, which is based on the estimated glomerular filtration rate (eGFR) rather than a calculation of the creatinine clearance (CrCl) [1]. Besides, several studies showed that both age and renal insufficiency (RI) were risk factors for venous thromboembolism (VTE) and bleedings. Appropriate screening for RI is therefore of major importance to optimize patient care in this population.

Aims: To evaluate the discrepancies in RI screening depending on the method used for renal function evaluation in a population of elderly RI patients.

Methods: Individual data from patients included in the IRIS study were analyzed (539 patients, age ≥ 70 yo). CrCl was calculated with the Cockcroft-Gault (CG) formula and eGFR was estimated with the MDRD formula. As CG is the most common method used to date in practice, it has been considered as the reference. MDRD eGFR was compared to CG CrCl, after conversion into the same units: mL min⁻¹.

Results: 1) The prevalence of severe RI was 25.97% with CG (CrCl ≤ 30 mL min⁻¹) as compared to 8.91% with MDRD (eGFR ≤ 30 mL min⁻¹/1.73 m²). 2) When converting MDRD eGFR into mL min⁻¹ to allow comparison, the difference was even slightly higher: 25.97% with CG CrCl and 7.42% with MDRD eGFR (difference -18.55%)

Conclusion: These findings demonstrate that using CG CrCl formula leads to: 1) a high prevalence of severe RI, especially since the unit used in CG are inconsistent as compared to the international definition (mL min⁻¹ instead of mL min⁻¹/1.73 m²). 2) a high prevalence of patients with a renal function below 30 mL min⁻¹ with 18.55% of falsely diagnosed patients with such an impaired renal function, and thus identified as contraindicated or not recommended for numerous anti-coagulant agents.

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PO633-WED

Association between red cell distribution width and risk of venous thromboembolism

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Background: Recently, an association between high values of red cell distribution width (RDW) and venous thromboembolism (VTE) has been observed. However, it is not known whether this association differs within various manifestations of VTE, nor if there is an interaction between RDW and established markers of thrombophilia on the risk of VTE.

Aims: 1) To investigate whether RDW is a marker of the risk of VTE; 2) to identify subgroups of patients in which the association between RDW and VTE is stronger; 3) to investigate a possible interaction between RDW and thrombophilia abnormalities.

Methods: We carried out a case-control study of 730 patients with a first objectively-confirmed episode of VTE (300 unprovoked and 430 provoked) consecutively referred to our Center between 2007 and 2013, and 352 healthy controls. Blood was taken for a thrombophilia work-up (antithrombin, protein C, protein S, factor V Leiden, prothrombin G20210A, antiphospholipid antibodies, factor VIII, homocysteinemia) and a complete blood count, including RDW, at least 3 months after VTE.

Results: Individuals with RDW above the 90th percentile ($> 14.6\%$) had a 2.5-fold increased risk of VTE compared to those with RDW below the 90th percentile, independently of age, sex, body mass index, other hematological variables and renal function (adjusted odds ratio: 2.52 [95%CI:1.42–4.47]). The risk was similar for unprovoked and provoked VTE, and higher in patients with pulmonary embolism (adjusted odds ratio 3.19 [95%CI:1.68–6.09]) than in those with deep vein thrombosis alone (2.29 [95%CI:1.22–4.30]). No interaction between high RDW and thrombophilia abnormalities on the risk of VTE was observed.

Conclusion: Our findings confirm RDW as an independent and easily available marker for stratification of the risk of VTE, which was particularly high in patients with pulmonary embolism.

Disclosure of Interest: None declared.

PO634-WED

Patient and doctor engagement: the missing link in preventing venous thromboembolism

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Background: General awareness of venous thromboembolism (VTE), risk factors and prevention is currently very low in Australia. Patient and their family engagement is critical to improving health care outcomes and reducing health care costs.

Aims: To evaluate consumer awareness of VTE risk and assess the importance of the patient point of view on VTE prevention.

Methods: In total 1018 Australian people completed a series of online VTE questions in June 2013. Participants requiring surgery or an extended hospital length of stay in the last 3 years, or be planning to have surgery or an extended in-hospital stay in the next 2 years were included. All participants were screened ensuring genuine responses. In the course of the survey VTE was explained to all research participants.

Results: Overall 2% of patients were concerned about VTE. Most (30%) patients were more uneasy about the effects of anaesthetic or in-hospital acquired infections. VTE risk was mentioned by a health care provider (HCP) in 36% of cases and was less likely raised in a public (28%) than a private (41%) hospital. It was also more likely to have been mentioned for elective surgery (44%) than for maternity patients (5%). Once participants were aware of the VTE risk, 84% were extremely or very likely to prefer to go to a hospital with 'best practice' VTE prevention. This was favoured even if it equated to inconvenience for visitors (42%), further travel (36%), expensive treatment (25%) or changing surgeons (22%).

Conclusion: These findings highlight the importance of active discussion between HCP, patients and their families to increase community VTE awareness. Appropriate risk assessment is key to implementing appropriate prophylactic measures. Patient education on their VTE personal risk profile, family history, use of preventative measures and compliance is critical to VTE risk reduction. Engaging clinicians and creating accountable institutions is key to minimising the burden of VTE.

Disclosure of Interest: Nondeclared.

PO635-WED

Epidemiology of thromboembolic disease in patients undergoing hepatectomy

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Background: Hepatectomy patients are at significant risk of venous thromboembolism (VTE). Pulmonary embolism (PE) rates have been reported to be as high as 6%. Concerns on the use of pharmacological prophylaxis due to post-operative liver dysfunction and bleeding remain an issue.

Aims: To determine the incidence of VTE following hepatectomy as well as the efficacy of prophylactic modalities used and associated bleeding complications.

Methods: In total, 265 patients were retrospectively reviewed between 2005 to 2014 that underwent hepatectomy for benign or confirmed malignancy. Demographic details, diagnosis, VTE risk, hospital length of stay (LOS), operative details, prophylactic modalities used and bleeding complications were collected and analyzed. Thrombotic events included the in-hospital and 1 year incidence of deep vein thrombosis (DVT), pulmonary embolism (PE) and portal vein thrombosis (PVT).

Results: Male to female ratio was 1.4:1 with a median age of 59 years (50–68.5) and LOS of 8 days (6–12). Median operative duration was 4 h (2.9–5.3). The majority (83.1%) of patients underwent surgery for malignancy. All patients received mechanical prophylaxis, 89.5% received unfractionated heparin (UH), 2.6% low molecular weight heparin (LMWH) and 7.9% UH followed by LMWH. 14.7% of patients on UH had a major bleed. The in-hospital thrombotic rate was 1.9%, 0.8% for PVT and 1.1% for non-fatal PE. At 1 year the incidence of thrombotic events was 3.1%, 0.8% for PVT, 0.4% for DVT and 1.9% for non-fatal PE. Post-hepatectomy after discharge VTE accounted for 50% of all events. Mortality at 30 days was 1.1%.

Conclusion: The in-hospital VTE rate was kept relatively low with use of prophylactic protocols. Late VTE events occurred after hospitalization with results revealing an almost two fold increase in PE at 1 year. These findings suggest that selective use of post discharge pharmacological prophylaxis post-hepatectomy in patients that are clearly identifiable as high risk may be beneficial.

Disclosure of Interest: None declared.

PO636-WED

Impact of weight change on risk of venous thromboembolism: the tromsø study

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Background: Obesity is a major risk factor for venous thromboembolism (VTE), but limited knowledge exists on whether weight change over time affects VTE risk.

Aims: To investigate the association between weight change and risk of incident VTE in a large prospective cohort of subjects recruited from the general population.

Methods: Participant data were collected from the Tromsø 4 (1994–95), 5 (2000–01) and 6 (2007–08) surveys. Subjects who attended two subsequent or all surveys were included. A total of 7189 subjects, aged 25–89, were followed from the median date between two subsequent visits until 2 years after the second visit or to the date of an incident VTE, death or migration. Subjects who attended all three surveys contributed with two different observations, yielding 11592 observations in total. Cox regression models were used to calculate risk of VTE across quintiles of

weight change (body weight second visit - body weight first visit). The middle quintile (0.1–2.2 kg weight gain) was used as reference. The effect of weight change independent of obesity status was assessed by adjustment for body mass index (BMI) at the time of the second visit.

Results: There were 101 incident VTE events during a median of 5.8 years of follow-up. Subjects in the highest weight change quintile (5.1–42.0 kg weight gain) had a 3-fold increased risk of VTE compared to the middle quintile (HR 2.96; 95% CI 1.26–6.96). Adjustment for BMI did not attenuate the risk estimates (HR 2.95; 95% CI 1.23–7.08). Subjects in the lowest quintile (56.6–2.6 kg weight loss) also had an increased risk of VTE (HR 2.23 95% CI 1.00–5.01). Subjects with BMI > 25 in the highest weight change quintile had a 4-fold increased VTE risk (HR 3.95; 95% CI: 1.37–11.43).

Conclusion: Our prospective cohort study implies that weight change, particularly weight gain, is a risk factor for VTE independent of obesity. Weight gain in overweight and obese subjects had an additive impact on the VTE risk.

Disclosure of Interest: None declared.

PO637-WED

Developing a case definition for venous thromboembolism-related deaths using death certificates

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Background: Death certificates are a chief data source for identifying deaths related to venous thromboembolism (VTE). Death certificates have two parts: causes directly related to death (four fields), and significant conditions (one multi-line field). Nosologists subsequently use ICD-10 codes based on text in these fields. The optimal case definition for surveillance of VTE-related death is unknown but may include data from text and ICD-10 data fields.

Aims: Develop a case definition for surveillance of VTE-related deaths by analyzing the contributing components of the text and ICD-10 code fields in the death certificate.

Methods: A broad set of ICD-10 codes (I26, I73, I74, I80, I81, I82, I87, O03, O04, O07, O08, O22, O87, and O88) was used to identify all deaths potentially related to VTE in Oklahoma County during January 1, 2010–May 31, 2014 from the Oklahoma State Department of Health. Text from the cause of death and significant conditions fields was compared to the ICD-10 fields to assess agreement.

Results: We identified 860 deaths potentially related to VTE using the above codes. Of these, 547 had evidence of being VTE-related in the text fields, 272 from the cause of death fields and 282 from the significant conditions field, with 7 having a VTE-related condition in both sections. Limiting the ICD-10 codes to I26, I80, I81, I82, I87, O22, and O88 resulted in identifying 557 individuals, of which 546 were true cases with confirmatory evidence from ≥ 1 text field, eight (1.5%) had a VTE-related code but no corresponding text, and three (0.5%) records were too nonspecific by code or text to determine if the event was VTE-related. The ICD-10 codes: I73, I74, O03, O04, O07, O08, O87 were excluded as being too broad to be reliably VTE-related.

Conclusion: We developed a case definition using ICD-10 codes I26, I80, I81, I82, I87, O22, and O88 that identified VTE-related deaths with only eight (1.5%) false positive cases and three (0.5%) inconclusive cases for VTE relation.

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PO638-WED

Case-fatality after pulmonary embolism and stroke in subjects with atrial fibrillation

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Background: Atrial fibrillation (AF) is a well-known risk factor for stroke and has recently been identified as a risk factor for pulmonary embolism (PE). To what extent AF affect case-fatality after PE or stroke has, however, previously not been investigated.

Aims: To investigate whether AF affected case-fatality after PE or stroke in a population based cohort.

Methods: Subjects ($n = 29\ 588$) were recruited from three surveys of the Tromsø study and followed from enrollment (1994–95, 2001–02 and 2007–08) to December 31, 2010. Incident events of AF, PE and stroke during follow-up were identified and validated. Information on potential confounders was obtained at baseline. Case-fatality rates (CFR) were calculated, and Cox-regression models with age as time scale were used to calculate hazard ratios (HRs) with 95% confidence intervals (CIs) for case-fatality of PE and stroke. The study was approved by the research ethicscommittee and informed written consent was obtained.

Results: There were 215 subjects with incident PE of which 34 associated with AF, and 1095 subjects with incident stroke of which 181 associated with AF, during follow-up. There was no difference in the 1-year CF rate between PE and stroke when adjusting for potential confounders (HR 1.01; 95%CI, 0.70–1.44). The 1-year crude CFR from PE was higher in subjects with AF associated PE CFR 0.45; 95% CI, 0.25–0.81) than in subjects without AF (IR 0.25; 95%CI, 0.18–0.35). Similarly, the 1-year crude CFR from stroke was higher in subjects with AF (CFR 0.40; 95%CI, 0.30–0.52) than for subjects without AF (CFR 0.30; 95%CI, 0.26–0.34). The apparent higher case-fatality caused by PE (HR 1.24; 95%CI, 0.48–3.18) or stroke (HR 0.99; 95% CI, 0.71–1.37) in subjects with AF disappeared after adjustment for sex and other potential confounders.

Conclusion: Our findings imply that PE and stroke display similar 1-year case-fatality, and that the presence of AF did not modulate the 1-year case fatality from PE and stroke when potential confounders were taken into account.

Disclosure of Interest: None declared.

PO639-WED

Hospital acquired thrombosis (HAT) in an Irish tertiary referral hospital

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Background: Venous thromboembolism (VTE) is a major cause of preventable morbidity and mortality in hospitalized patients. VTE prevention has been recognized as the most important patient safety issue internationally. Prior to introduction of a National VTE prevention programme in the UK, it was estimated that there were more than 25,000 hospital deaths annually from VTE. Despite its proven efficacy and safety the uptake of thromboprophylaxis (TP) internationally has

been disappointing, Ireland, like many countries has yet to implement mandatory risk assessment and TP for hospitalised patients.

Aims: The aim of this study was to estimate the period prevalence of HAT in a tertiary referral centre and to examine whether VTE risk assessment and TP were implemented in these cases.

Methods: We examined the 15 weeks period prevalence of radiologically confirmed VTE in adult patients. Patient notes were examined to determine if a risk assessment for VTE had been performed during admission and if appropriate TP was initiated.

Results: 100 patients were diagnosed with VTE over this time period. 72 were admitted with VTE while 28 developed VTE as an inpatient. 20 of the 72 patients that were admitted with VTE had a hospital admission in the past 3 months. Overall, HAT was felt to be a possible diagnosis in 48 of the 100 cases. Formal VTE risk assessment was not performed in 29 (60%) cases of likely HAT. 65% of these 48 ($n = 31$) did not receive any or appropriate TP during their hospitalisation. Despite TP, 17 (35%) patients developed VTE. The majority, 39 cases (80%) of HAT occurred within 28 days of hospitalization.

Conclusion: Hospitals without a comprehensive strategy for implementation of a VTE prevention program and ongoing monitoring of adherence to such a program likely continue to have a significant rate of HAT and consequently a significant rate of preventable morbidity and mortality.

Disclosure of Interest: None declared.

PO640-WED

Prevalence and risk factors for pulmonary embolism in patients with acute exacerbation of copd: results of a large collaborative study

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Background: Pulmonary embolism (PE) may be frequent complication of chronic obstructive pulmonary disease (COPD) triggering acute dyspnoea in these patients. Since PE can lead to similar presentation to acute exacerbation of COPD, it is often difficult to distinguish between these two entities. However, only a few studies with a limited sample size have assessed the prevalence of PE and the presence of potential risk factors for PE in patients with a suspect acute exacerbation of COPD.

Aims: To evaluate the prevalence of PE in a large cohort of patients with a suspect acute exacerbation of COPD; to compare characteristics of patients with and without a diagnosis of PE. To compare the mortality rate in patients with and without PE.

Methods: Thirty-three Italian centers were involved. All the patients with a suspect acute exacerbation of COPD undergoing angio-computed tomography for a suspect of PE from 1st January to 31st December 2011 were includible in the study. Patients' baseline characteristics, and data on clinical, laboratory and radiological characteristics will be registered. Furthermore, information on concomitant disease and on potential risk factors for PE will be collected. Characteristics and prognosis of patients with and without PE will be compared.

Results: A total of 1043 patients were included. PE was diagnosed in 132 patients (mean prevalence 12.7% (95% CI 10.7, 14.9%). Female gender (OR 2.38, 1.60, 3.53), previous VTE (OR 2.10, 1.21, 3.63), age > 75 years (OR 1.78, 1.17, 2.73) and hypocapnia at blood gas analysis (OR 1.73, 1.16, 2.59) were significantly associated with an increased risk of PE at multivariate analysis. Mortality was not significantly higher in PE patients (6.1% vs 5.0%).

Conclusion: Our large multicenter study confirmed the high PE prevalence in patients with a suspect acute exacerbation of COPD. Female gender, older age, previous VTE and hypocapnia at blood gas analysis were significantly associated with an increased risk of PE in this population.

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PO641-WED

Risk of first and recurrent venous thrombosis in individuals treated with antibiotics

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Background: Previous register studies suggest a role for transient infectious disease in the etiology of venous thrombosis (VT, encompassing deep vein thrombosis [DVT] and pulmonary embolism [PE]).

Aims: To study whether individuals who receive antibiotic treatment (as a proxy for infectious disease), have an increased risk of first or recurrent VT.

Methods: 4731 patients with a first VT were included in the MEGA-study and followed for a median of 5.9 years for recurrence. Information on antibiotic use was obtained via linkage to SFK data (Dutch Foundation for Pharmaceutical Statistics). We used the self-controlled case-series (SCCS) method to study the risk of *first* VT after a prescription of antibiotics. A SCCS analysis is limited to cases, so non-transient confounders do not play a role. The risk of *recurrent* VT after antibiotic use was studied by means of time-dependent Cox regression, with correction for age and sex.

Results: 2547 VT patients could be individually linked to SFK data, in whom 127 first events occurred during antibiotic use. The risk of VT was increased during antibiotic treatment: (incidence rate ratio [IRR] 5.6 (95%CI;4.6–6.8)). Clinical presentation of PE, and to a lesser extent DVT, may at first be misdiagnosed as an infection. Based on information from discharge letters we excluded 13 individuals in whom such misclassification played a role, leaving an IRR of 5.0 (95%CI, 4.0–6.1). We further restricted the analysis to DVT (with possibly less misclassification) resulting in an IRR of 3.8 (95%CI, 2.8–5.2). The IRR of DVT in patients receiving antibiotics for urinary tract infections (least chance of misclassification) was 3.3 (95%CI, 1.9–5.7). Antibiotic use was associated with a 2.1-fold (95%CI;1.1–4.1) increased risk of *recurrent* VT compared with no use.

Conclusion: Individuals who receive antibiotics have an increased risk of both first and recurrent VT. Misclassification of VT symptoms as infection may lead to overestimation of the association.

Disclosure of Interest: None declared.

PO642-WED

Inhospital mortality among clinical and surgical inpatients recently diagnosed with venous thromboembolic disease

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Background: Venous thromboembolism (VTE) is the most common cause of preventable mortality in hospitalized patients, and pulmonary embolism (PE) is responsible for 5–10% of all hospital deaths.

Aims: To estimate the hospital mortality in hospitalized patients who developed VTE during hospitalization.

Methods: Prospective cohort of incident cases of VTE developed during hospitalization in patients over 17 years, in a period of 7 years in a tertiary hospital care in Buenos Aires. Inhospital global mortality and fatality rate of inpatients with VTE was calculated.

Results: The cumulative incidence of VTE was 1.8% (95% CI 1.77–1.93%), representing 1.32% (95% CI 1.23–1.41%) in the subgroup of surgical patients and 2.1% (95% CI 1.9–2.2%) in clinical inpatients. The overall hospital mortality was 2.4% (95% CI 2.35–2.53); being 3.95% (95% CI 3.78–4.12) in clinical inpatients and 1.15% (95% CI 1.06–1.23) in surgical patients. The death in patients who had developed VTE, represented between 4–7% of hospital deaths, and it increases with age in both clinical and surgical patients.

Conclusion: In Argentina, there are few data of hospital mortality in patients with VTE. This information is useful when assessing the need for resources for prevention, diagnosis and treatment in inpatients.

Disclosure of Interest: None declared.

PO643-WED

Low prevalence of symptomatic venous thromboembolism after hip and knee arthroplasty at siriraj hospital

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Background: In major orthopedic surgery, medical and mechanical thromboprophylaxis (Thp) are recommended routinely in western countries. However, medical Thp is not widely used in Thailand because of the belief of a low incidence of postoperative venous thromboembolism (VTE) particularly symptomatic VTE (sVTE) and the controversy regarding the risk-benefit and cost-effectiveness.

Aims: To determine the prevalence of sVTE in patients undergoing knee or hip arthroplasty (KA or HA) without medical Thp at Siriraj hospital

Methods: Prospective observational study included cases aged above 18 years who underwent KA or HA between Feb, 2013 and Nov, 2014. Cases taking antithrombotic drug before surgery or having an underlying bleeding disorder were excluded. Data of postoperative sVTE during admission and 3 months after surgery were collected. The sVTE included deep-vein thrombosis (DVT) detected by duplex ultrasonography (or computed tomographic venography) and documented pulmonary embolism (PE).

Results: A total of 896 cases (741 women) undergoing KA ($n = 714$) or HA ($n = 182$) had a median age of 68 years (range 21 to 94). Two women (67&89 years) developed symptomatic DVT of operated leg at 45 and 16 days after surgery, respectively. There was no PE. Thus, the prevalence of sVTE was 0.22% (95%CI 0.04 to 0.90). Tranexamic acid

was given in 68.9% of cases pre- and/or post-operatively. Preoperative thrombotic risks included cancer ($n = 32$), estrogen use ($n = 4$), a history of VTE ($n = 2$), obesity ($n = 163$), congestive heart failure (CHF; $n = 6$) and varicose veins ($n = 14$). Both VTE cases had no thrombotic risk. Postoperative calf muscle exercise was employed in all cases. Median days of postoperative immobilization were 2 days (range 0 to 74). Three cases died within 3 months postoperatively due to meta-static cancer, DRESS syndrome and CHF.

Conclusion: Prevalence of symptomatic VTE in Thai patients undergoing major orthopedic surgery was lower than in Western reports. Medical thromboprophylaxis might not be recommended in all Thai patients.

Disclosure of Interest: None declared.

PO644-WED

The prevalence and diagnostic characteristics of patients with antiphospholipid antibodies in chronic thromboembolic pulmonary hypertension

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Background: The antiphospholipid syndrome (APS) is a risk factor for chronic thromboembolic pulmonary hypertension (CTEPH). Diagnosis can be made either by the dilute Russell viper venom time (dRVVT) or by quantification of anticardiolipin or beta2-glycoprotein antibodies. The APS necessitates more aggressive anticoagulation in the early post-pulmonary thromboendarterectomy period.

Aims: We sought to describe the prevalence and diagnostic test characteristics of antiphospholipid antibodies among patients with CTEPH referred for pulmonary thromboendarterectomy (PTE).

Methods: We examined consecutive patients referred to UCSD for PTE between January 1, 2012 and June 30, 2014. All patients underwent hypercoagulability testing including dRVVT and anticardiolipin antibody screening. The prevalence of APS was determined. Comparisons between patients with and without APS were made. A t-test was used to compare continuous variables and a chi square test was used for dichotomous variables.

Results: Of the 404 patients who underwent PTE, APS was identified in 36.3% (SD $\pm 4.7\%$) of all patients. The dRVVT and anticardiolipin antibodies were both positive in only 19.5% of APS cases. The dRVVT was positive in 87.9% of cases while anticardiolipin antibodies were present in 33.5% of cases. Patients with APS were younger (mean age 50.4 vs. 53.6, $P = 0.03$), but there was no significant difference in gender or pre-operative pulmonary vascular resistance compared to patients without APS. Post-operative complications including re-thrombosis and bleeding complications were similar between both groups.

Conclusion: Presence of antiphospholipid antibodies is common among CTEPH patients referred for PTE. Given the prothrombotic state of APS, standard of care has resulted in a more aggressive anticoagulation regimen in the immediate post-PTE period. Identification of these antibodies in this patient population therefore becomes essential to lowering the risk for rethrombosis while not exposing patients to excessive risk for bleeding.

Disclosure of Interest: None declared.

PO645-WED

Predictors of venous thromboembolism recurrence, adjusted for treatments and interim exposures: a population-based case-cohort study

Heit JA¹, Lahr BD², Ashrani AA¹, Petterson TM² and Bailey KR²¹Internal Medicine; ²Health Sciences Research, Mayo Clinic, Rochester, USA**Background:** Predictors of VTE recurrence remain uncertain.**Aims:** To identify independent predictors of VTE recurrence, adjusted for treatments and interim exposures.**Methods:** Using Rochester Epidemiology Project resources, all Olmsted County, MN residents with objectively-diagnosed incident VTE over the 13-year period, 1988–2000, who survived ≥ 1 day were followed for first objectively-diagnosed VTE recurrence. For all patients with recurrence, and a random sample of all surviving incident VTE patients, we reviewed the complete medical records in the community and collected demographic and baseline characteristics, treatments and interim VTE risk factor exposures. In a case-cohort study design, demographic, baseline, treatment and interim characteristics were tested as potential predictors of VTE recurrence using time-dependent Cox proportional hazards modeling.**Results:** Among 1262 incident VTE patients, 306 developed recurrence over 6440 person-years. Five-year recurrence rates, overall and for cancer-associated, idiopathic and non-cancer secondary VTE, were 24.5%, 43.4%, 27.3% and 18.1%, respectively. In multivariable analysis, interim hospitalization $\pm$ surgery (hazard ratio [HR] = 6.6 and 5.2, respectively), active cancer (HR = 3.4), pregnancy (HR = 12.9), central venous catheter (HR = 4.3) and respiratory infection (HR = 1.5) were associated with increased hazards of recurrence, and warfarin (HR = 0.4) and aspirin (HR = 0.6) were associated with reduced hazards. Adjusting for treatments and these interim risk factors, male sex (HR = 1.3), baseline active cancer (HR = 2.6) and heparin resistance (HR = 1.6) were independently associated with increased hazards of VTE recurrence over the entire follow-up period, while the hazards of recurrence for patient age, chronic lung disease, leg paresis, prior superficial vein thrombosis and idiopathic VTE varied over the follow-up period.**Conclusion:** Baseline and interim exposures can stratify VTE recurrence risk and may be useful for directing secondary prophylaxis.**Disclosure of Interest:** None declared.

Venous thromboembolism – therapy III

PO646-WED

Dabigatran or warfarin in the treatment of acute venous thromboembolism in antiphospholipid syndrome

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uncertainty whether the non-vitamin K antagonist oral anticoagulants are effective for the treatment of VTE in patients with antiphospholipid syndrome (APS).

Aims: To analyze the efficacy and safety of dabigatran vs. warfarin in patients with VTE and APS.**Methods:** We reviewed data from the pivotal RE-COVER and RE-COVER II studies on the treatment of acute VTE and from RE-MEDY study on the extended treatment of VTE. Thrombophilia testing was not mandatory but whenever performed before the trial, we captured all diagnoses of APS, defined as one positive test for lupus anticoagulant and/or for cardiolipin antibodies combined with symptomatic, objectively verified VTE. Patients that were enrolled in RE-MEDY after being in RE-COVER or RE-COVER II were only counted once and only as long as they remained on the original anticoagulant. Symptomatic, objectively verified VTE recurrence or fatal VTE, major bleeding, clinically relevant non-major bleeding (CRNMB) and any bleeding events were recorded.**Results:** Altogether, 6868 patients were randomized and 1871 (27.4%) were tested for APS. Of these, 151 (8.6%) had a positive test for APS; 71 were treated with dabigatran and 80 with warfarin. Mean age was 48 years and 36% were female (similar in the 2 treatment arms). There were 3 (4%) recurrent VTE events on dabigatran and 4 (5%) on warfarin (hazard ratio [HR] 0.43; 95% confidence interval [CI], 0.08–2.4); 5 in RE-COVER studies and 2 in RE-MEDY. Major bleeding occurred in 1 patient (1%) on dabigatran and 2 (2.6%) on warfarin (HR 0.46; 95% CI, 0.04–5.4), major and CRNMB in 6 (9%) and 14 (18%), respectively (HR 0.53; 95% CI, 0.20–1.4) and any bleeding in 14 (20%) and 31 (40%), respectively (HR 0.50; 95% CI, 0.26–0.95).**Conclusion:** There was no evidence for decreased efficacy of dabigatran compared to warfarin in the treatment of VTE in patients with APS. Bleeding events were reduced with dabigatran.**Disclosure of Interest:** S. Schulman Grant/Research Support from: Boehringer Ingelheim, Baxter, Octapharma, S. Goldhaber Grant/Research Support from: Bristol-Myers Squibb, Boehringer Ingelheim, Consultant for: Boehringer Ingelheim, Merck, Bristol-Myers Squibb, Medscape, C. Kearon Consultant for: Boehringer Ingelheim, A. Kakkar Consultant for: Sanofi Aventis, Pfizer, Eisai Inc, GSK, Bayer Healthcare, Boehringer Ingelheim, Daiichi Sankyo, Bristol-Myers Squibb, Speaker Bureau of: Sanofi Aventis, Pfizer, Eisai Inc, GSK, Bayer Healthcare, Boehringer Ingelheim, Daiichi Sankyo, Bristol-Myers Squibb, S. Schellong Consultant for: Bayer Healthcare, Boehringer Ingelheim, GlaxoSmithKline, Sanofi, H. Eriksson Consultant for: Boehringer Ingelheim, Pfizer, Bayer Healthcare, Leo Pharma, Bristol-Meyers Squibb, E. Schueler Consultant for: Boehringer Ingelheim, M. Feuring Employee of: Boehringer Ingelheim, J. Kreutzer Employee of: Boehringer Ingelheim.

PO647-WED

Multicenter prospective study of treatment and risk factors for unusual site thrombosis: trust registryMa K¹, Wells P², Guzman C³, Anderson D⁴, Blostein M⁵, Hirsch A⁶, Kovacs MJ⁷, Lazo-Langner A⁸, Rodger M⁹, Tagalakakis V¹⁰ and Kahn S¹⁰¹Medicine, Division of Hematology, McGill University, Montreal;²Clinical Epidemiology, Ottawa Hospital Research Institute, Ottawa;³Clinical Epidemiology, Lady Davis Institute, Montreal;⁴Medicine and Clinical Epidemiology, Dalhousie University, Halifax;⁵Medicine, Division of Hematology; ⁶Medicine, Respiratory Division, Jewish General Hospital, Montreal;⁷Medicine, Division of Hematology; ⁸Medicine and Clinical Epidemiology, Division of Hematology, University of Western Ontario, London;⁹Medicine and Clinical Epidemiology, Ottawa Hospital Research Institute, Ottawa;¹⁰Medicine and Clinical Epidemiology, Jewish General Hospital, Montreal, Canada

Background: Unusual site deep vein thrombosis (USDVT) is an uncommon form of venous thromboembolism (VTE) with significant heterogeneity in pathophysiology and clinical features depending on the site affected. While anticoagulation treatment is generally accepted, there is a paucity of data on optimal USDVT treatment.

Aims: The TRUST registry study aimed to characterize the epidemiology, optimal treatment and outcomes associated with USDVT.

Methods: This prospective registry included 152 patients recruited from May 2008 to October 2012 at 4 Canadian tertiary care centers. Suggested treatment of USDVT was standardized across centers: initial heparin followed by 6 months of therapeutic anticoagulation. Baseline demographics were collected and follow-ups were performed at 6 months, 1 and 2 years.

Results: There were 97 (63.8%) cases of splanchnic, 33 (21.7%) cerebral, 14 (9.2%) jugular, 6 (3.9%) ovarian and 2 (1.3%) renal vein thrombosis. Mean age was 52.9 years and most (74.3%) cases were symptomatic. The main risk factors for cerebral vein thrombosis were recent hospitalization, active malignancy and hormone exposure and for jugular vein thrombosis, immobility and CVCs. Previous VTE, family history and sex were generally not common risk factors for USDVT. Of 72 (47.4%) patients who had thrombophilia testing as part of clinical care, 24 (33.3%) were diagnosed with new thrombophilia. Of the 138 patients who were seen in follow-up, 66 (47.8%) patients completed at least 6 months of anticoagulation. In total, there were 4 (2.9%) cases of recurrent VTE and 22 (15.9%) deaths; 19 from progressive malignancy. There were no fatal bleeding events.

Conclusion: Despite half of USDVT patients being treated with < 6 months of anticoagulation, rate of VTE recurrence during 2 years follow-up was low and anticoagulant treatment of USDVT appears safe. Thrombophilia testing was common and thrombophilia prevalence was quite high. Further research is needed to determine the optimal investigation and management of USDVT.

Disclosure of Interest: None declared.

PO648-WED

The within patient effect of statin use on coagulation in vitamin k antagonist users: a crossover studyRein NV^{1,2}, Biedermann JS^{3,4}, Bonafacio SM², Kruip MJ^{3,4}, Lijfering WM^{1,2,5} and van der Meer FJ²¹Eindhoven Laboratory for Experimental Vascular Medicine;²Department of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden;³Star-Medical Diagnostic Center;⁴Department of Hematology, Erasmus University Medical Center, Rotterdam;⁵Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands

Background: Previous studies suggest that statins affect coagulation in vitamin K antagonist (VKA) users, but results are conflicting, as not all studies took confounding fully into account.

Aims: To determine the immediate and long-term effect of statins on coagulation in patients treated with phenprocoumon or acenocoumarol.

Methods: A crossover study was performed on patients from 2 anticoagulation clinics from the Netherlands who initiated treatment with a statin between 2009–2013. Patients were excluded who used concomitant drugs that interact with VKAs or were hospitalised during the study period. The VKA dosage (mg/day) and INR after statin initiation was compared with the last VKA dosage/INR measured before statin was started. Immediate (< 21 days) and long-term effects (at 6 and 12 weeks) were calculated with a paired student t-test.

Results: 738 Patients on VKA were included, of which 435 were phenprocoumon users (mean age 70 years; 60% men) and 303 were acenocoumarol users (mean age 69 years, 58% men). After start of statin use, the immediate phenprocoumon dosage was 0.04 mg day⁻¹ (95% CI, 0.02 to 0.07) lower. At 6 and 12 weeks, these dosages were 0.09 (95% CI, 0.04 to 0.14) and 0.12 mg day⁻¹ (95% CI, 0.06 to 0.19) lower as compared with the dosage before statin use. For acenocoumarol these numbers were 0.04 mg day⁻¹ (95% CI, 0.01 to 0.07) lower (immediate effect), and 0.08 (95% CI, 0.06 to 0.19) and 0.14 mg day⁻¹ (95% CI, 0.00 to 0.37) lower, at 6 and 12 weeks after statin was initiated, respectively. No apparent effect was seen on INR in all analyses. Limitation: As the population base consisted of VKA users only, we cannot exclude that results are due to drug-drug interactions.

Conclusion: Statin treatment was associated with a decrease in VKA dosage in both phenprocoumon and acenocoumarol users, which suggests that statins have anticoagulant properties.

Disclosure of Interest: None declared.

PO649-WED

Rivaroxaban vs. vitamin k antagonist in provoked deep venous thrombosis - which treatment is better for the patient?Groarke E¹, Courtney W¹, Conway J¹, Conway E¹, Bourke D¹, Saunders J², Watts M³ and O'Keeffe D¹¹Department of Haematology, University Hospital Limerick,

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Background: Deep venous thrombosis (DVT) is a common medical condition that particularly occurs associated with an underlying provoking factor. Current guidelines recommend 3 months of anticoagulant therapy for all patients with a provoked DVT. Traditionally, the agent of choice has been oral vitamin K antagonists (VKA), in combination with sub-cutaneous low molecular weight heparin. VKA require intensive monitoring particularly during the initiation of anti-

coagulation, and have a relatively narrow therapeutic window. Direct oral agents (DOACs) have recently been licensed for the treatment of DVT.

Aims: To compare the cost, safety, and patient clinic visit requirements for rivaroxaban vs. VKA.

Methods: Prospective data was collected on new patients with provoked DVT who received rivaroxaban for three months between November 2013 and December 2014. This data was compared to an age and sex matched group of historical patients who received a VKA in our unit before November 2013.

Results: 24 patients received rivaroxaban during the study period. This data was matched to a comparable historical group treated in our unit. Average age was 49.8 in VKA group vs. 48 in rivaroxaban group with 3:7 M/F ratio. 79% of DVTs were below knee. No significant bleeds occurred in either group. 37.5% of patients on VKA spent < 60% of time in the therapeutic range. They spent an median time of 17% in supratherapeutic and 15% in subtherapeutic range. VKA patients required significantly more clinic visits. (14.58 visits vs. 2.92). The overall cost to the health service per patient for 3 months treatment was E260.68 for VKA compared to E273.30 for rivaroxaban. This does not include the cost to the patient of multiple clinic visits.

Conclusion: For provoked DVT rivaroxaban treatment resulted in less outpatient attendance, with similar overall drug costs to VKA, and safer anticoagulation.

Disclosure of Interest: None declared.

PO650-WED

Warfarin dosing and time to therapeutic international normalized ratio in patients with hypercoagulability syndromes

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Background: Hypercoagulability syndromes (HS), which are genetic or acquired syndromes with a defined abnormality involving a component of the coagulation cascade, are well known causes of venous thromboembolism (VTE). Warfarin is the most common medication used for the treatment of VTE. It is not known if a difference exists in the dose and time needed to achieve therapeutic anticoagulation between patients with and without HS.

Aims: To analyze the difference in the dose of warfarin and the time required to reach the target therapeutic international normalized ratio (INR) between patients with and without HS who are managed for VTE.

Methods: An institutional review board approved case control study was conducted at our hospital. Inpatient and outpatient charts from January 2002 to December 2012 were reviewed. A total of 622 patients met our inclusion criteria. Two groups were compared: Group 1 were VTE patients with a secondary International Classification of Diseases, Ninth Revision, code for HS and Group 2 were patients with VTE without a secondary HS diagnosis, i.e. control group.

Results: A total of 125 cases in Group 1 and 497 in Group 2 were analyzed. Univariate ($P < .2$) and multivariate analyses ($P \leq .05$) were performed with the primary aim of comparing Group 1 to Group 2. The dose of warfarin required to reach therapeutic INR in Group 1 was higher (50.7 ± 17.6 mg) compared to Group 2 (41.2 ± 17.7 mg). The difference was statistically significant ($P < .001$). The total number of days required to reach therapeutic INR in Group 1 was (8.9 ± 3.5 days) as compared to (6.8 ± 2.9 days) in Group 2. The difference was statistically significant ($P < .001$).

Conclusion: Our study suggests that patients with HS require a higher dose of warfarin and a longer time to reach therapeutic INR. This

study suggests that more attention is needed when managing patients with HS to ensure proper and timely anticoagulation.

Disclosure of Interest: None declared.

PO651-WED

Safety of stopping anticoagulant treatment for cancer-associated venous thromboembolism after reaching complete remission from cancer

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Background: After diagnosis of cancer-associated venous thromboembolism (caVTE), guidelines recommend extended anticoagulation or until complete remission of cancer has been achieved. In clinical practice, anticoagulation is often discontinued in patients who have achieved complete remission of cancer, although this practice has never been evaluated in prospective studies.

Aims: To determine the risk of recurrent VTE after discontinuation of anticoagulation for caVTE in patients who achieved complete remission of cancer.

Methods: A cohort study of consecutive patients diagnosed with caVTE at the Leiden University Medical Center between April 2002 and January 2010.

Results: Of the 358 patients diagnosed with caVTE, 196 patients survived the first 6 months after VTE diagnosis, of who 79 had a complete remission of cancer. Anticoagulation was discontinued in 67 of them and in 12 patients anticoagulation was continued because of another indication. Among these 67 patients, 10 symptomatic VTE recurrences were diagnosed during a cumulative follow-up of 310 years resulting in an incidence rate (IR) of 3.2/100 PY (95%CI 1.5–5.9). Seven of these recurrences (70%) occurred among patients with a cancer relapse and 7 of the 15 patients (47%) with a cancer relapse or a new cancer diagnosed during follow-up developed recurrent VTE. In the 49 patients who remained cancer-free, the IR of recurrent VTE was 1.2/100 PY (95%CI 0.2–3.5). Among the 117 patients who were not cured from cancer, the IRs of recurrent VTE under anticoagulation, recurrent VTE after cessation of anticoagulation and of anticoagulation-associated major bleeding were 6.3/100 PY (95%CI 2.0–15), 12/100 PY (95%CI 5.8–22) and 11/100 PY (95%CI 5.1–21) respectively.

Conclusion: Our data underline the recommendation to discontinue anticoagulant therapy for caVTE after patients have reached complete remission. A cancer relapse during follow-up however seems to be a strong risk factor for recurrent symptomatic VTE.

Disclosure of Interest: None declared.

PO652-WED

Prolonged use of lmwh during pregnancy and bone safety

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Background: It is uncertain whether long-term low-molecular-weight heparin (LMWH) decreases bone mineral density.

Aims: To determine if prolonged use of dalteparin or enoxaparin during pregnancy leads to persistent loss of bone mineral density (BMD).

Methods: BMD was measured using dual-energy X-ray absorptiometry (DEXA) in women, who received long-term LMWH during pregnancy ($n = 92$), and women without any use of LMWH ($n = 60$) on average 5 years (range 1–12 years) after the delivery. Every women also filled a questionnaire with basic characteristics and several questions related to BMD-related factors.

Results: BMD in spine was significantly lower in the intervention group than in the control group (1.22 g cm^{-2} vs. 1.27 g cm^{-2} , $P = 0.017$), but no difference existed in femoral neck (0.99 g cm^{-2} vs. 1.01 g cm^{-2} , $P = 0.473$). Anyhow no correlation between BMD and dose, duration nor type of the LMWH, and age of women existed. Changes in BMD were not explained by age at menarche, calcium-intake, physical activity, smoking, alcohol use, duration of the lactation. Strong positive correlation with BMD and body mass index existed. BMD also correlated positively with the duration of combined contraception pill use. The controls have used combined contraception pills significantly longer than the intervention group, because of the high risk of thrombosis.

Conclusion: Though BMD in spine was lower in intervention group than in control group it did not appear to be related to LMWH-use. The higher body mass index and longer combined contraception pill use may explain the situation partially. We conclude that LMWH even with prolonged use seems to be safe with regard to bone health.

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PO653-WED

Practice variation in the management of distal dvt between gps and vascular medicine physicians: a french survey of physician practices

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Background: Distal deep-vein thromboses (DVT) are infra-popliteal DVT without concomitant pulmonary embolism. Though as frequent as proximal DVT, their management is debated, country and even physician-dependant.

Aims: To assess whether the management of distal DVTs varies between general practitioners (GP) and vascular medicine physicians (VMP) in France and to describe DVT patient's ambulatory pathway.

Methods: Clinical practice telephone survey conducted from January to June 2013 among a random selection of GP and VMP working in Languedoc-Roussillon region (France).

Results: 111 GP and 56 VMP agreed to participate (refusal rate: 19%). In case of suspicion of DVT, GP refer their patients to a VMP, a cardiologist or a radiologist in 69%, 23% and 6% of cases respectively. In case of DVT, GP manage their patients alone, in association with a VMP or do not manage treatment in 18%, 63% & 18% of cases respectively. In case of joint therapeutic management, VMP use to start, assess and stop anticoagulants and compression stocking treatments (> 75% of cases) whereas GP monitor anticoagulation (> 80% of cases). In case of distal DVT, there was no difference between MG and VMP in terms of use of anticoagulants (94% vs. 92%) and intensity of anticoagulation (full dose: 99% vs. 100%). However, duration of anticoagulation differs: GP modulate less frequently anticoagulation in presence of a transient risk factor (55% vs 84%, $P < 0.05$) and according to the deep calf or muscular veins character of DVT (5% vs. 36%, $P < 0.05$). GP also tend to treat more frequently distal DVT as proximal DVT (47% vs 7%, $P < 0.05$).

Conclusion: In Languedoc-Roussillon, treatment of isolated distal DVT is similar between GP and VMP and involves systematic use of full dose of anticoagulants. Duration of anticoagulants differs and almost half of all GP do not modulate DVT treatment according to

anatomical location of DVT (proximal, distal, deep calf or muscular veins) and its provoked or unprovoked character.

Disclosure of Interest: None declared.

PO654-WED

Improving evidence-based decisions in VTE treatment: simulation in continuing education

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Background: Evidence-based anticoagulant therapy is widely underused in patients with VTE, resulting in preventable morbidity, mortality, and healthcare costs.

Aims: This study was conducted to determine if an online, simulation-based continuing medical education (CME) intervention could improve performance of cardiologists in managing patients with VTE.

Methods: The intervention consisted of two cases presented in a platform that allowed learners to choose from numerous lab tests, diagnoses and treatments. The clinical decisions made by the participants were analyzed using an artificial intelligence engine, and instantaneous or delayed clinical guidance was provided based on current evidence and expert recommendation. Participant decisions were collected after clinical guidance and compared with each user's baseline data using a 2-tailed paired T-test to provide P values for assessing the impact of education on clinical decisions.

Results: The assessment sample consisted of 504 cardiologists. As a result of clinical guidance, significant improvements were observed: • 12% improvement in orders for appropriate diagnostic tests for a patient who presents with symptoms of deep vein thrombosis (DVT) (78% post intervention vs 70% baseline, $P < 0.001$) and a 23% improvement for a patient with symptoms of pulmonary embolism (PE) (66% vs 55%; $P < 0.001$). • 21% improvement in appropriate selection of anticoagulant therapy for a patient with DVT (51% vs 42%; $P = 0.007$), and a 46% increase for a patient with PE (21% vs 46%; $P < 0.001$). 72% increase in orders for outpatient follow-up for a patient with DVT (29% vs 50%; $P < 0.001$) and a 110% increase for a patient with PE (42% vs 20%; $P < 0.001$) 119% increase in orders for compression stockings for a patient with DVT (21% vs 46%; $P < 0.001$).

Conclusion: This study demonstrated that simulation-based CME can improve evidence-based practices of cardiologists, and suggests that this type of intervention can improve outcomes for patients with VTE.

Disclosure of Interest: None declared.

PO655-WED

Risk of recurrent venous thromboembolism in patients with a first episode of unprovoked pulmonary embolism: a pre-specified subgroup analysis from the padis pe randomized trial

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Background: Patients with a first episode of unprovoked pulmonary embolism (PE) have a high risk of recurrent venous thromboembolism (VTE) after anticoagulation is discontinued.

Aims: To identify subgroups of patients who had a benefit of extended anticoagulation.

Methods: In a double-blind randomized trial, 371 patients who had completed 6-month anticoagulation for a first unprovoked PE were randomized to receive either warfarin or placebo for an additional 18-month period. Patients were subsequently followed for a period of 24 months after study drug discontinuation. The primary outcome was the composite of recurrent VTE or major bleeding during the treatment period. The secondary outcome was the composite during

the entire study period. The effect of extended anticoagulation on outcomes was analysed in pre-specified subgroups of patients.

Results: During the treatment period, the primary outcome occurred in 6 of 184 patients (3.3%) in the warfarin group and in 25 of 187 (13.5%) in the placebo group (hazard ratio [HR], 0.22; 95% confidence interval [95%CI], 0.09 to 0.55; $P = 0.001$). During the entire study period, the composite outcome occurred in 33 patients (20.8%) in the warfarin group and in 42 (24.0%) in the placebo group (HR, 0.75; 95%CI, 0.47–1.18; $P = 0.22$). Results of pre-specified subgroup analyses (age, sex, comorbidities, anticoagulation quality, thrombophilia, residual lung perfusion defects, persistent right ventricular dysfunction or D-dimer < 250 microgr/L) on the composite outcome during the treatment and entire study periods were all consistent with the overall treatment effect.

Conclusion: Extending for a further 18 months an initial 6-month anticoagulation in patients with a first unprovoked PE markedly reduced the risk of the composite of recurrent VTE and major bleeding, but this benefit was lost after treatment discontinuation. No heterogeneity was observed in pre-specified subgroups of patients. (ClinicalTrials.gov number NCT00740883).

Disclosure of Interest: None declared.

PO656-WED

New oral anticoagulants (NOACs) in vte treatment: will monitoring diminish their cost-effectiveness?

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Background: New oral anticoagulants (NOACs) have shown comparative efficacy and safety to vitamin K antagonists (VKAs) in the treatment of venous thromboembolism (VTE) and the prevention of recurrences. A major advertised advantage of NOACs is their fixed dose precluding monitoring. However, monitoring might be preferred, especially in subgroups like elderly patients and is assumed to affect the cost-effectiveness of NOACs in these groups.

Aims: We determined the main cost drivers of the cost-effectiveness of NOACs and the data availability regarding subgroups that might require monitoring by reviewing appraisals from health technology assessment (HTA) bodies in The Netherlands (ZiN) and the UK (NICE).

Methods: We reviewed HTA submissions and appraisals for NOACs from ZiN and NICE focusing on the economic evidence appraisal conclusions, the cost drivers and the subgroups investigated.

Results: From NICE, appraisals were available for rivaroxaban and dabigatran. In general, rivaroxaban and dabigatran showed similar efficacy and costs, and were considered cost-effective as the incremental cost effectiveness ratio (ICER) to VKAs remained under £20,000 per quality-adjusted life-year gained. However, VKA monitoring costs were a main cost driver with the ICER increasing above the threshold with reduced VKA monitoring costs. The manufacturer submissions did not include results for relevant subgroups (e.g. patients with reduced renal function or elderly patients with DVT or PE), which were underrepresented in clinical trials. In the Netherlands, rivaroxaban was appraised and considered cost-effective. Again, monitoring was considered a cost driver and no subgroup results were shown.

Conclusion: The cost-effectiveness of NOACs in VTE treatment and prevention will likely diminish when monitoring costs for NOACs will be introduced. As a consequence, this might influence the uptake of NOACs for VTE patients who require monitoring.

Disclosure of Interest: None declared.

PO657-WED

Community hospitals are using novel oral anticoagulants and more aspirin as venous thromboembolism (VTE) prophylaxis following total hip and knee replacement

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Background: VTE after total hip and knee replacement surgery (THR and TKR) is a major threat to patient (pt) safety. Prophylaxis agents with high potency to prevent VTE are available but many practitioners, particularly orthopedic surgeons, have not prescribed them due to bleeding concerns. Registry data from 2001–2004 revealed a high rate of warfarin use (56%) followed by LMWH (44%). Approval of novel oral anticoagulants (NOACs) and the Association of American Orthopedic Surgeons guideline recommending aspirin on equal footing as high potency agents likely have changed prescription patterns.

Aims: To examine VTE inpatient prescription patterns, particularly use of aspirin and NOACs among pts undergoing THR and TKR in a large network of community hospitals.

Methods: We identified consecutive pts age 66 + who underwent elective THR and TKR in 28 community hospitals within the database of the Institute of Health Metrics, a nonprofit quality improvement and data management organization. We analyzed inpatient prophylaxis for any pt with a hospital stay of ≥ 2 days through nursing barcode scanning, which confirms receipt of medication. We also collected pt demographics from administrative data and comorbidities from nurse chart review.

Results: A total of 3786 surgeries met our eligibility criteria. 1089 underwent THR and 2697 underwent TKR. Surgical volume varied; 22 hospitals performed 30 + surgeries and 12 performed 100 +. Mean pt age was 74; 62% were female; 94% were white. HTN, DM, CAD were present in 73%, 21%, and 18% of the pts, respectively. Mean length of stay was 3.2 days. Of the 3786 pts, 3751 (99%) received some form of drug prophylaxis. Warfarin was the most often used (39%) followed by enoxaparin (34%), aspirin (11%), NOACs (10%), and fondaparinux (5%).

Conclusion: VTE prophylaxis was nearly universal in this community-based cohort. There has been a shift toward more aspirin use and also adoption of NOACs. In our future work, we plan to report how these changes affect VTE and hemorrhage rates.

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PO658-WED

Global adherence to American college of chest physicians guidelines for thromboprophylaxis after major orthopedic surgery. have we improved? a systematic review of the literature

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Background: Increased risk of deep vein thrombosis (DVT) and pulmonary embolism (PE) following major orthopedic surgery (MOS) is

well described in the literature. In order to reduce this risk, ACCP guidelines for thromboprophylaxis include recommendations for these patients. Currently, there is a gap between clinical practice and the implementation of these recommendations.

Aims: The aim of this study is to identify and compare the global adherence to the last 4 editions of ACCP guidelines (6th, 7th, 8th and 9th ed.) for thromboprophylaxis after MOS.

Methods: A systematic review of literature was performed following PRISMA methodology. Ovid, PubMed and Embase databases were used. Studies reporting adherence to ACCP guidelines for thromboprophylaxis after MOS during the last 10 years were included. Primary outcomes were: adherence to ACCP guidelines for global, in-patient and post-discharge thromboprophylaxis. Proportions of adherence were calculated for each outcome.

Results: Of 3993 titles identified, a total of 14 studies reporting adherence to the 6th, 7th or 8th editions of ACCP guidelines were included. No studies evaluating the 9th ed. were found. The global adherence to the recommendations for MOS was 54.3%. Adherence to in-hospital and post-discharge thromboprophylaxis was 90.1% and 66.72%, respectively. The 6th edition showed the highest rate of adherence (59.58%). During the last 10 years, a statistically significant ($P < 0.005$) reduction in the rates of compliance was found.

Conclusion: We observed a significant decrease in the rates of adherence to the recommendations when comparing 2001 (6th) and 2008 (8th) editions. As adherence to post-discharge thromboprophylaxis is significantly lower, further efforts are required to increase this rate and reduce the risk of DVT and PE. Reasons that could explain these results are discussed.

Disclosure of Interest: None declared.

PO659-WED

Incidence of thromboembolism in korean multiple myeloma patients with thalidomide induction chemotherapy; comparison previous data in Korea

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Background: The patients with multiple myeloma (MM) shown high incidence of thromboembolism (TE) because of hypercoagulability. The incidence is higher when patients received immunomodulatory agent. The incidence of thromboembolic event has been reported to be high (10–58%) in Caucasian patients with MM who received thalidomide, but the incidence has been known to be relatively low in Asian patients.

Aims: In 2010, TE incidence of 360 MM patients was checked and confirmed relatively low incidence in Korea. We intended to evaluate the incidence of TE in newly diagnosed MM patients who received thalidomide as induction chemotherapy and compare the results with previous study.

Methods: We retrospectively reviewed 169 MM patients who received thalidomide between 2000 and 2014 at our institution. All of the patients were below 65 years old at the time of diagnosis and received thalidomide as induction chemotherapy. We surveyed the incidence of TE and use of prophylactic anticoagulation therapy in these patients. The results was compared with our previous data.

Results: The patients who had prophylactic anticoagulation therapy were forty-six (27.2%). Among them, 44 patients took aspirin. Six patients (3.6%) developed thromboembolism. The sites of TE were extremities (4), lung (1) and celiac os (1). One of these patient had prophylactic treatment with aspirin and one patient received low molecular weight heparin. Not a single clinical parameter including gender, stage, subgroup and period of thalidomide administration predicted a

TE development ($P > 0.05$). Compared to the previous data which analyzed Korean MM patients who received thalidomide in various settings, incidence of TE during thalidomide induction chemotherapy seemed to be similar (3.6% vs 3.9%).

Conclusion: The incidence of TE in Korean MM patients who receive thalidomide as induction chemotherapy is low. Not a single factor predicted development of TE. The genetic characteristics in Asian MM patients should be further defined.

Disclosure of Interest: None declared.

PO660-WED

Thromboprophylaxis in a patient with morbid obesity

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Background: Obesity is a risk factor for venous thromboembolism (VTE) in hospitalized patients. There is evidence in obese patients that the risk of VTE exceeds risks from complications of the surgical procedure in bariatric surgery, however presently there is no clear guidance for VTE thromboprophylaxis in these patients.

Aims: To describe our experience of thromboprophylaxis in a patient weighing 383 kgs (BMI 120 kg m⁻²).

Methods: A 25 year old man was referred to Clinical Haematology for advice on thromboprophylaxis during hospital admission. Comorbidities were morbid obesity (weight 383 kg, BMI 120 kg m⁻²), hypothyroidism, essential hypertension and chronic lower limb lymphoedema. He was given 50 iu kg⁻¹, once daily dose of tinzaparin as thromboprophylaxis while in hospital with peak anti-Xa levels monitored.

Results: This patient underwent bariatric surgery and further plastic surgery procedures, using this thromboprophylactic regimen, with peak anti-Xa levels between 0.38 iu L⁻¹ to 0.41 iu L⁻¹, and no documented VTE or bleeding complications.

Recommendations for VTE prophylaxis in obese patients are not clear, other regimens have been described, stratified on the basis of BMI. For example enoxaparin 40 mg with BMI ≤ 50 kg m⁻² or 60 mg with BMI > 50 kg m⁻² every 12 h, with peak anti-Xa levels (0.2–0.4 IU mL⁻¹ $\pm 10\%$). Bleeding was not associated with a high anti-Xa concentration. High-dose thromboprophylaxis reduces the risk of VTE in morbidly obese inpatients. Extended thromboprophylaxis is recommended following discharge.

Conclusion: We describe a thromboprophylaxis regimen in a morbidly obese patient, using once daily administration of low-molecular-weight heparin (LMWH). This is the first report in a patient of this weight (383 kg, BMI 120 kg m⁻²). The administration of LMWH, in various dosing regimens has been described; however, randomized clinical trials to compare these in obese patients are necessary.

Disclosure of Interest: None declared.

PO661-WED

Coagulation tests for detection of VKA therapy insufficiency in patients with acute deep vein thrombosis

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Background: The risk of DVT recurrence during VKA therapy can achieve 16%.

Aims: We aimed to determinate the group of patients with insufficient of warfarin therapy.

Methods: Patients with unprovoked DVT ($n = 34$) were treated with UFH 150 U kg⁻¹ 3 times per day from 1st to 10th day, Warfarin 5 mg per day starting from day 6. Dose adjustment of warfarin according to INR was performed after day 15. Blood samples were collected on 10 and 15 days. Global coagulation tests were performed: thrombin generation test TGT (EPT, normal range 700–1470 nM*min) and thrombodynamics assay (the spatial clot growth rate V, normal range 20–30 $\mu\text{m min}^{-1}$). INR and D-dimer also were measured. Data presented as median or median (25%, 75%).

Results: On day 10th patients had D-dimer level 273 (134, 370) ng mL⁻¹. After withdrawal of heparin part of patients (7/34, 24%) increased D-dimer level, indicating that formation of new fibrin deposits occurred in significant amount, despite of an anticoagulation. We divided patients into 2 groups at 15th day: Group 1 with elevated D-dimer level (D-dimer > 400 ng mL⁻¹) and Group 2 without D-dimer elevation (D-dimer < 400 ng mL⁻¹). There was no significant difference between these two groups by INR (2.4 and 3.5 respectively). Thus, an adequate anticoagulant effect by INR was observed in both groups. There was no significant difference by TGT also (650 and 400 nM*min respectively). V was significantly higher in the Group 1 (21 $\mu\text{m min}^{-1}$) compared to Group 2 (17 $\mu\text{m min}^{-1}$), $P < 0.05$. Most of patients in the Group 1 had V within the normal range, indicates an insufficient anticoagulant effect of warfarin. ROC-analysis was used for definition the ability of V to predict D-dimer increase, AUC = 0.72. $V < 17.8 \mu\text{m min}^{-1}$ showed high negative predictive value 95.5%.

Conclusion: Thus, thrombodynamics V has a high (95.5%) negative predictive value for increase of D-dimer level in patients on warfarin therapy within a 1st week of unprovoked DVT treatment.

Disclosure of Interest: None declared.

PO662-WED

Biomarkers for prediction of reoccurrence of venous thromboembolism

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Background: Venous Thromboembolism (VTE) is a significant health concern because of its high death rate, and it is characterized by high rates of reoccurrence. It would be helpful clinically to have biomarkers that allow early identification of patients with high or low risk of reoccurrence for diagnosis and therapy assessment.

Aims: The objective of this work is to determine if biomarkers can predict the risk for VTE reoccurrence.

Methods: We evaluated steady state levels of FVIII, D-dimer, fibrinogen concentration and additional components of an extensive thrombophilia in groups of patients after having only one thrombotic episode, compared to patients having multiple thrombotic episodes, and control patients having no thrombotic episodes. Then, a microfluidic flow assay was used to measure and distinguish between the fibrin generation and platelet function in these cohorts of patients. A Thromboelastograph (TEG) was also used to assess their fibrinolytic abilities.

Results: The steady state values of D-dimer and fibrinogen levels and FVIII levels were elevated in patients having reoccurring thrombotic episodes compared to patients in single occurrences. The microfluidic assay revealed a 28.7% increase in fibrin generation in patients with reoccurring episodes more than single occurring patients.

The TEG also revealed a decrease in fibrinolytic activity in reoccurring patients compared to single episode patients.

Conclusion: Our predictions have implications for management of thrombosis. We found that reoccurring thrombosis patients had ele-

vated levels of plasma FVIII, D-dimer and fibrinogen. An *in vitro* microfluidic flow assay showed that reoccurring thrombotic patients also had an increase in their fibrin generation and they had an impaired fibrinolysis as confirmed by the TEG

Disclosure of Interest: None declared.

Vitamin K antagonists III

PO663-WED

Medication adherence of novel oral anticoagulant vs. warfarin

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Background: It was hypothesized that rivaroxaban patients may perform worse than warfarin due to difference in medication costs and relatively infrequent monitoring recommended.

Aims: To compare the medication adherence and treatment persistence of patients on warfarin vs. rivaroxaban for prophylaxis and treatment of thromboembolism.

Methods: Retrospective case control study of patients on warfarin and rivaroxaban from the period of September 2009 to October 2014 was performed. Patients on rivaroxaban were extracted and matched in 1:2 against warfarin users based on indication. A total of 144 warfarin and 72 rivaroxaban users were analysed. Primary outcome of adherence was determined with Medication Possession Ratio (MPR), while treatment persistence was determined by outpatient clinic appointment gaps. Pertinent patient demographic characteristics (age, race, gender, duration of treatment, concomitant medications and residence type) were also collected and analysed.

Results: The medication possession ratio of warfarin patients was worse than rivaroxaban patients by 9.49% ($P < 0.001$, 95% CI: 5.51% > 13.49%). Also, there were more warfarin patients who had gaps in treatment persistence compared to those prescribed rivaroxaban (12, 8.3% vs 1, 1.4%). Other documented factors suspected to affect medication adherence were also analysed by multivariable linear regression. Among them, age (coef: 0.179, 95% CI: 0.059–0.229) and duration of drug use (coef: 0.149, 95% CI: 0.046–0.252) turned out to be significant determinants.

Conclusion: Contrary to hypothesis, patients on rivaroxaban appeared to be more adherent to their prescribed oral anticoagulant as demonstrated by a better MPR and better treatment persistence. Factors including age and duration of drug use are also found to be potential risk factors for non-adherence in this selected group of patients, and should be considered at the point of prescribing by physicians and tailored counselling by pharmacists.

Disclosure of Interest: None declared.

PO664-WED

Risk of major bleeding in patients treated with vitamin K antagonists or aspirin. a systematic review and meta-analysis

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Background: Prior meta-analysis and observational studies have suggested that the bleeding risk associated with vitamin K antagonists (VKA) targeting an INR between 2–3 and aspirin have the same risk bleeding risk, independent of the indication.

Aims: To provide the odds ratios (ORs) of major bleeding (primary outcome), intracranial bleeding and fatal bleeding episodes, and overall mortality associated with the use of VKA or ASA.

Methods: We conducted a systematic review Ovid MEDLINE, Embase and the Cochrane Central Register of Controlled Trials (RCT), from 1946 to May 28th 2014. RCTs reporting bleeding rates in adult patients randomized to a VKA [mean target International Normalized Ratio (INR) between 2–3 without concomitant ASA], or to ASA alone (< 325 mg daily), with at least 3 months of follow-up were included. Random effects ORs and their associated 95% confidence intervals (CIs) were calculated.

Results: Fifteen RCTs reporting the outcome of 2549 participants treated with VKA alone and 2471 treated with ASA alone were included; most common conditions evaluated were non-valvular atrial fibrillation (five trials) and heart failure (three trials). The use of vitamin K antagonists was associated with an increased risk of major bleeding random effects pooled OR 1.76 (95% CI 1.24–2.48; I^2 10.8%). All-cause mortality was similar between those treated with VKA and ASA, random effects pooled OR 0.95 (95% CI 0.8–1.13; I^2 0%) whereas the risk of intracranial bleeding random effects pooled appeared to be increased in those receiving VKA OR 1.74 (95% CI 0.83–3.62; I^2 0%) but not the risk of fatal bleeding OR 1.25; (95% CI 0.4–3.92; I^2 0%).

Conclusion: Contrary to prior reports, our results suggest that the risk of major bleeding with the use VKA is higher compared to patients treated with ASA alone, with a trend towards an increased risk of intracranial bleeding.

Disclosure of Interest: None declared.

PO665-WED

Impact of point-of-care INR monitoring on anticoagulant control during treatment with vitamin K antagonists in patients monitored by a specialized anticoagulation clinic: a population based cohort study

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Background: Prior studies have shown that INR monitoring using a point-of-care (POC) device improves quality of treatment with vitamin K antagonists (VKA) in self-monitoring patients. However, POC monitoring is also being introduced for monitoring regular patients by anticoagulation clinics.

Aims: To determine the effect of POC INR monitoring by nurses on anticoagulant control in regular patients on VKA.

Methods: POC monitoring of regular patients by nurses was implemented in a specialized anticoagulation clinic. Time in therapeutic range (TTR) was the primary outcome and was calculated using Rosendaal's linear interpolation method. We compared TTR the year before (laboratory, $n = 1973$) and after (POC, $n = 1959$) introduction of POC monitoring. Secondary outcomes were percentage of INR results within therapeutic range, INR testing frequency and incidence of clinical events (major bleeding, ischemic stroke, hospitalization and all-cause mortality). Incidence rates and hazard ratios of clinical events were compared during laboratory and POC monitoring using the log-rank test and Cox proportional hazard models respectively. This study was not subject to the medical research involving human subjects act and was approved by the local medical ethics committee.

Results: Median TTR (80.9% vs. 77.9%, $P < 0.001$) and percentage of INR results within therapeutic range (70.1% vs. 66.8%, $P < 0.001$) were significantly lower and INR testing frequency significantly higher

(19.7 vs. 21.0, $P < 0.001$) during POC monitoring. Compared with laboratory monitoring, POC monitoring was not associated with an increased risk of major bleeding (HR 0.93, 95%CI 0.56 to 1.52), ischemic stroke (HR 0.92, 95%CI 0.42 to 2.02), hospitalization (HR 0.94, 95%CI 0.79 to 1.12) or all-cause mortality (HR 1.00, 95%CI 0.78 to 1.29) both unadjusted and adjusted for age.

Conclusion: Compared with laboratory monitoring, POC monitoring is associated with significantly poorer anticoagulant control in regular patients on VKA, although short-term clinical outcome is similar.

Disclosure of Interest: None declared.

PO666-WED

Multi-dose drug dispensing as a tool to improve medication adherence: a crossover study

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Background: Multidose drug dispensing (MDD) is a dosing aid that provides patients with disposable bags containing all drugs intended for one dosing moment. It is believed that medication adherence increases when using MDD instead of individually dispensed drugs, but these studies are based on self reported data and results may be biased due to socially acceptable answers.

Aims: To determine the effect of MDD on medication adherence with an objective outcome, in non-compliant patients on vitamin K antagonists (VKAs), and to determine whether MDD is equally effective as instructing patients.

Methods: Patients treated with VKAs are monitored on a regular basis and the time in therapeutic range (TTR) reflects the stability of treatment. TTR was used as a surrogate marker for adherence (i.e. objective outcome). Non-compliant patients were defined on the basis of a TTR < 75% 6 months prior to the start of MDD. The TTR before and after start of MDD were compared within patients to estimate changes in adherence after starting MDD. Non-compliant patients usually receive letters or are contacted by telephone by nurses from the anticoagulation clinic to improve adherence. To analyze whether such instruction works equally well as MDD, non-compliant patients who did not start MDD were matched to the MDD users to compare their TTR increase over time.

Results: 83 Non-compliant patients started using MDD during VKA treatment. The TTR increased 13% (95% CI 6% to 21%) within 1 month after starting MDD and remained stable during the next 5 months. The TTR of MDD- as compared with non-MDD patients increased 10% more (95% CI -1% to 22%) within 1 month, but was similar after 6 months.

Conclusion: While MDD was associated with higher adherence within 1 month, MDD and instruction of patients are associated with equal improvement of drug adherence over a 6 month period.

Disclosure of Interest: None declared.

PO667-WED

CYP2C9 polymorphism controls phenprocoumon metabolism resulting in severe bleeding events

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Background: Vitamin K antagonists (VKA) such as phenprocoumon and warfarin are widely used to reduce the risk of thrombosis, pulmonary embolism and stroke. In particular they are applied for primary or secondary prevention of clotting in complex congenital heart disease including univentricular circulation. Dosing of VKA is complicated due to a large interindividual variation for therapeutic anticoagulation, thereby resulting in major bleeding events. While genotype-guided dosing of warfarin is internationally recommended using well-established dosing algorithms, similar evidence for phenprocoumon, is still limited.

Aims: We report on a 25 year old patient who received phenprocoumon following a modified Fontan repair for a univentricular heart.

Methods: Additional risk factors for thrombosis had been a fenestration in the Fontan tunnel, an open pulmonary trunk as well as a pre-existing thrombus in the V. brachiocephalica. One year after onset of phenprocoumon the patient presented with an upper gastrointestinal bleeding, and the INR was found to be above 8. As initial INR measurements had been stable, the patient had stopped testing 8 month earlier. A cranial CT scan was performed due to headache, nausea and vertigo and revealed intracranial hemorrhage in the area of a previous cranial abscess. Furthermore, the patient sustained a muscular hemorrhage in the right calf. Phenprocoumon was stopped immediately, but repetitive high doses of vitamin K reduced INR values only for several hours. It took three weeks for the INR values to reach normal ranges again.

Results: Genetic testing for the CYP2C9 alleles *2 and *3 as well as the VKORC1 variants G3673A, C6484T and G9041A revealed the homozygous variant genotype CYP2C9*3/*3.

Conclusion: The present case supports the impact of CYP2C9 genetics - particularly in a homozygous variant manner - as risk factor for bleeding events under phenprocoumon therapy due to impaired drug metabolism.

Disclosure of Interest: None declared.

PO668-WED

Increased risk for major bleeds after minor bleeds is determined by unknown persistent common risk factors

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Background: The most common side effect of vitamin K antagonists (VKAs) is bleeding. Literature shows that in patients using VKAs a minor bleed is a warning sign for a subsequent major bleed, independent of the quality of anticoagulation. Minor bleeds in one anatomical location are unlikely to cause a major bleed in another location, which implies that the association is due to common causes (e.g. age, comorbidity). It is not known what these common causes are and whether these are persistent (such as diabetes) or transient.

Aims: To examine whether common causes for minor and major bleeds during VKA treatment are transient or persistent.

Methods: Included were patients who started VKA treatment at the Leiden Anticoagulation Clinic between 2003 and 2013. Patients were considered exposed starting at time of a minor bleed until three months after this bleed. Whether common causes were transient or persistent was studied in two analyses. First, a Cox model with time-dependent variables which was adjusted for known common causes (age, sex, INR target range, antidiabetic-, antihypertensive-, antiplatelet drugs and non-steroidal anti-inflammatory drugs). As a Cox model does not adjust for unknown persistent common causes, a case-crossover (CCO) study was also performed. A CCO corrects for all known and unknown persistent common causes as patients are compared with themselves, but does not adjust for transient ones.

Results: 26,130 Patients were included and followed for 61,672 years in which 7194 minor and 913 major bleeds occurred. The adjusted Cox model indicated that patients with minor bleeding were at 2.5 fold (95% CI 1.9–3.3) increased risk of major bleeding in the next 3 months. The CCO gave risk estimates around unity (OR 0.9, 95% CI 0.5–1.5), indicating that common causes were persistent.

Conclusion: Unknown persistent common causes explain the association between minor and major bleeds. Further studies are needed to determine their nature.

Disclosure of Interest: None declared.

PO669-WED

Accuracy of a warfarin maintenance dose predictor after completion of the 10 MG initiation nomogram

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Background: A prediction formula for the warfarin maintenance dose after completion of 10 mg initiation nomogram was proposed in 2009 (maintenance dose [in mg] = 2.5 + 10% of the first week cumulative dose - INR value at Day 8 + 1.5 if INR was below 2.0 at Day 5). This formula defined the mean warfarin dose over the last 10 days of the first month of treatment as maintenance dose of warfarin. The developed formula has not been validated.

Aims: We sought to determine if use of the warfarin maintenance dose calculator in patients in whom we utilized the 10-mg initiation warfarin nomogram for acute VTE, accurately predicts the maintenance dose at month 2 and 3 after start of treatment.

Methods: A retrospective cohort of outpatients with VTE treated with warfarin during the period July 2010 to May 2012 at the Ottawa Hospital Thrombosis Unit were started on the 10-mg warfarin initiation nomogram and subsequently had their dose managed by a pharmacist with the assistance of Dawn software. We report on those patients who had received warfarin for at least 3 months with a target INR between 2 and 3 and more than 65% time in therapeutic range (TTR). We determined if the mean warfarin doses these patients received in month 2 and 3 of their treatment were similar to the “warfarin maintenance dose calculator” dose.

Results: Of 286 patients, 76 had < 65% TTRs leaving 210 patients for analysis. The Pearson correlation coefficient was 0.84 ($P < 0.0001$) between the doses of warfarin prescribed to patients in month 2 and 3 of their treatment and the maintenance doses of warfarin calculated for these patients using the prediction formula. Mean and Standard deviation of the difference between these two doses were also calculated (0.97 mg and ± 1.02 mg, respectively) and were not clinically significant.

Conclusion: The existing prediction formula for Warfarin maintenance dose is accurate and can be used to predict the maintenance dose in months 2 and 3 of Warfarin treatment.

Disclosure of Interest: None declared.

PO670-WED

A national anticoagulant management service provided by community pharmacies in new zealand achieves a high level of anticoagulant control which is safe and convenient for patients

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Background: A community pharmacy based anticoagulant management service using point of care (POC) testing (CoaguChek, Roche Diagnostics) and online decision support software (DSS) (INR Online) was introduced in New Zealand in 2011. Pharmacists can provide the service if they have completed a 1 day training course and gained accreditation. The service is supported by funding from the Ministry of Health.

Aims: To assess anticoagulant control (Time in range (TTR)), testing frequency, patient adherence with testing and frequency of adverse events for this service.

Methods: An audit for the period 2012 to 2014 using data collected from the online support software. The test date, INR result, warfarin dose and adverse events were recorded at each visit.

Results: 5065 patients on warfarin had at least 2 INR tests during the audit (1/1/12 to 31/12/14). 126 pharmacies provided the service. Overall TTR was 74.1% (based on 1.8million days on treatment and 106,500 INR tests), with 9.9% above and 15.9% below the range. 75.1% of patients had a TTR > 60% and 17.2% had a TTR > 90%. Median test interval was 18.9 days (interquartile range (IQR) 13.6–24.3) with a median of 1.6 tests/month (IQR 1.25–2.24). Testing adherence was high with 74.6% of INR tests performed on the recommended date and 89.1% within 3 days, only 5.3% of tests were more than 7 days overdue. 3441 bleeding episodes were reported (82% minor, 16% moderate, 2% major); one bleed for every 540 days on warfarin.

Conclusion: Pharmacists in community pharmacies are able to achieve a high level of anticoagulant control using POC testing and DSS. The internet based data collection allows accurate audit of results. Anticoagulant control was good (TTR 74.1%) with 75% of patients with a TTR above the recommended 60%. The incidence of major bleeding was low. Testing adherence was high, in part due to follow-up procedures and patient email reminders. This type of service offers patients easy access to warfarin monitoring with rapid results and immediate treatment advice.

Disclosure of Interest: P. Harper Shareholder of: INR Online Ltd (Warfarin management software), I. McMichael: None declared, D. Griffiths: None declared, C. Hill: None declared.

PO671-WED

Recurrent thrombotic events after discontinuation of vitamin k antagonist treatment for splanchnic vein thrombosis: a multicenter retrospective cohort study

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Background: It is generally recommended that patients with splanchnic vein thrombosis (SVT) should receive a minimum of 3 months of anti-coagulant treatment. However, since little information on the long-term risk of recurrent thrombotic events is available, the optimal duration of secondary prevention remains unknown.

Aims: To evaluate the risk of venous and arterial thrombosis after discontinuation of vitamin K antagonist (VKA) treatment in SVT patients.

Methods: Retrospective information from a cohort of SVT patients treated with VKA and followed by 37 Italian Anticoagulation Clinic, up to June 2013, was collected. Only patients who discontinued VKA treatment and did not receive any other anticoagulant drug were enrolled in this study. Thrombotic events occurred during follow-up were centrally adjudicated.

Results: Ninety patients were included (median age 50 years, 52% males). According to the etiology of the thrombosis, 33 patients had unprovoked SVT, 27 SVT secondary to transient risk factors, and 30 SVT secondary to permanent risk factors. During a median follow-up of 1.6 years, 6 venous thrombotic events and 1 arterial thrombotic event were documented, for an incidence rate of 3.3 (95% CI, 1.6–6.9) per 100 patient-years (pt-y). The recurrence rate was highest in the first year after VKA discontinuation (8.2 per 100 pt-y, 95% CI, 3.7–18.3) and in patients with permanent risk factors (10.2 per 100 pt-y, 95% CI, 4.2–24.4). In particular, liver cirrhosis significantly increased the risk of recurrence (HR 7.9, 95% CI, 1.8–35.9).

Conclusion: The rate of recurrent vascular events, including arterial and venous thrombotic events, after SVT is not negligible, at least in some patient subgroups. Our findings need to be confirmed in larger prospective studies.

Disclosure of Interest: None declared.

PO672-WED

Patients with vitamin k antagonist (VKA) resistance: does the identification of VKORC1 mutations help predicting the maintenance dosage?

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Background: Patients requiring unusual high doses of VKA are rare and the management of such patients is challenging.

Aims: On behalf of the GEHT, we conducted a prospective multicenter study to identify genetic and non-genetic causes of "VKA resistance" and to evaluate the impact of these factors on phenotype severity.

Methods: Patients referred for identification of a genetic cause of VKA resistance were eligible in the present study if they received a VKA daily dose equal or greater than a dose-threshold of 10 mg-warfarin, 40 mg-fluindione or 6 mg-acenocoumarol (two-fold mean maintenance dosage in *VKORC1/CYP2C9* wild-type patients). A comprehensive standardized questionnaire was prospectively fulfilled by prescribers (demographic, clinical, comedications, environmental data). Genetic analysis included *VKORC1* coding region sequencing and *VKORC1* (-1639G>A), *CYP2C9* (*2/*3) and *CYP4F2* (p.Val433Met) SNP genotyping. We defined a resistance index as the ratio VKA observed dose / VKA dose-threshold. The potential influence of genetic and non-genetic factors was analysed by univariate and multivariate analysis using linear models.

Results: Of the 266 analysed patients, at least one mutation was detected in the *VKORC1* coding region in 73 (27.4%) patients. Some mutations were recurrent: p.Val66Met (*n* = 22), p.Asp36Tyr (*n* = 11), p.Arg12Arg (*n* = 9), p.Leu120Leu (*n* = 12), 4 patients being compound heterozygotes. Seven mutations had never been reported. No association was found between non-genetic variables and resistance index. The specific location of the mutations within the *VKORC1* structural and functional domains significantly influenced the resistance index. Especially, patients carrying mutations in/close to the putative warfarin binding interfaces had higher resistance indexes than non-carriers.

Conclusion: Despite a wide observed inter-individual variability in phenotype/genotype results, this pharmacogenetic approach might allow helping physicians in escalating VKA doses or proposing alternative oral anticoagulant therapy.

Disclosure of Interest: None declared.

PO673-WED

Similar performance of hasbled, CHADS2 and CHA2DS2VASC scores in bleeding risk prediction: do we need a bleeding score?

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Background: In the management of atrial fibrillation (AF) patients, CHADS2 score has been suggested to firstly stratify stroke risk,

CHA2DS2VASc to further stratify low-risk patients and HASBLED for bleeding risk, requiring clinicians to face with 3 scores. Bleeding risk scores have only a modest performance in predicting bleeding and most risk factors for stroke and major bleeding are “shared” risk factors

Aims: In this study we examined distribution of bleeds in each class of these scores to compare their predictive ability for bleeding events.

Methods: We prospectively studied 1085 AF patients (37.8%female; median age 75 years, follow-up 4697 patient-years). We stratify patients in relation to the 3 indicated scores.

Results: The average CHADS2 score was 2.2 ± 1.3 ; CHA2DS2-VASc was 3.7 ± 1.6 and HASBLED was 2.2 ± 1.0 ; 88 major bleeds were recorded (rate 1.78x100patient-years) and stratified according to the scores(table).

Score	CHAD n(%)	CHADSVASC n(%)	HASBLED n(%)
0	4/79 (5.1)	0/27	2/42 (4.8)
1	18/247 (7.3)	3/63 (4.8)	13/207 (6.3)
2	26/317 (8.2)	9/144 (6.2)	32/458 (7.0)
3	12/224 (5.4)	24/254 (9.4)	31/276 (11.0)
4	21/172 (12.2)	20/241 (8.3)	8/80 (9.1)
5	7/39 (17.9)	12/202 (5.9)	2/14 (14.3)
6	0/7	14/105 (13.3)	0/0
7	na	6/42 (14.3)	0/0
8	na	0/6	na
9	na	0/1	na

Predictive ability of HASBLED was 0.569 (0.507–0.631; $P = 0.030$), of CHADS2 was 0.559 (0.494–0.623; $P = 0.041$), of CHA2DS2VASc was 0.555 (0.493–0.616; $P = 0.047$).

Conclusion: The number of bleeds increases with the increase of the score for all models, with similar predictive ability for bleeding risk. HASBLED seems not to add useful information to identify high risk patients, and stroke stratification scores could be sufficient for tailoring treatment.

Disclosure of Interest: None declared.

PO674-WED

Low incidence of bleeding events in a “REAL WORLD”, vitamin k antagonist-treated patient population: results from the start-register

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Background: START-Register is an Italian, independent, inception-cohort, observational, database aimed at prospectively recording the clinical history of patients starting an anticoagulant treatment in “everyday clinical practice” and monitored in anticoagulation clinics (ACC).

Aims: In this report we analyze the incidence of major bleeds (MBs) during follow-up in patients treated with vitamin K antagonists (VKAs).

Methods: Patients’ demographic and clinical data were collected as electronic file on the web-site of the START-Register; the incidence of adverse events were recorded

Results: Presently, 7888 patients (53.0% males; median age: 74 years [interquartile range 65–80]) have been enrolled in the registry, 86% receiving VKAs and 14% Direct Oral Anticoagulants (DOACs); 61% of patients are treated for Non Valvular Atrial Fibrillation (NVAf), 29% for Venous Thromboembolism (VTE), and the remaining for other indications. We examined the 5731 pt/y follow-up currently available in 4637 patients on VKAs (3733 pt/y in NVAF, 1431 pt/y in VTE and 568 pt/y other indications). 82 MBs were recorded (1.5% pt/y in NVAF, 1.1% pt/y in VTE and 1.5% pt/y in other indications). The intracranial haemorrhages were 21 [15 in patient with NVAF and 4 in patients with VTE (rate 0.4 pt/y for all)]. MB events were more frequently in patients with moderate renal impairment ($P = 0.03$). No significant differences were detected in relation to age or other risk factors between patients with or without major bleeds.

Conclusion: The incidence of MBs was relatively low in an inception cohort of real life patients anticoagulated for NVAF or VTE and monitored in ACC, much lower than that recorded in the warfarin arms of recent clinical trials with DOACs in NVAF and also in VTE patients. A good monitoring quality is associated with a lower risk of bleeding in VKA-treated patients.

Disclosure of Interest: None declared.

PO675-WED

The start-register (survey on anticoagulated patients register): focus on patients treated for non-valvular atrial fibrillation

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Background: START-Register is an Italian, independent, inception-cohort, observational, database aimed at prospectively recording the clinical history of patients starting an anticoagulant treatment (vitamin K antagonists -VKAs or direct oral anticoagulant – DOACs) in “everyday clinical practice” and monitored in anticoagulation clinics (ACC).

Aims: In this report we give a cross section of non valvular atrial fibrillation (NVAf) patients.

Methods: Prospective data were collected as electronic file on the web-site of the START-Register. The follow-up is conducted to record: quality of treatment (for patients on VKAs), bleeding complications, thrombotic events, and the onset of any type of associated disease

Results: At this time 4833 NVAF patients (males 54%; median age 74 years; IQR 64–82; one-third had > 80 years) have been enrolled; 84.2% received VKAs and 15.8% DOACs (introduced only very recently in our National Health System). History of hypertension was recorded in 80% of patients, diabetes in 21%, history of previous thromboembolic event 14%; 5.3% had severe ($\text{CrCl} < 30 \text{ mL min}^{-1}$)

and 39.5% moderate renal failure. The follow-up was available for 68% of VKAs patients (3733 pt/y) and for 595 treated with DOACs (365 pt/y). During follow-up, major bleeds (MBs) were 58 (1.5% pt/y; 3 fatal) and 9 (2.5% pt/y, none fatal) in VKAs and DOACs patients, respectively. MBs were intracranial in 15 (0.4% pt/y) and 2 (0.5% pt/y), and gastrointestinal in 12 (0.4 pt/y) and 4 (1.1% pt/y) in VKA and DOACs patients, respectively. About 25% of MB occurred in the first 3 months of treatment.

Conclusion: In conclusion, these results show a low incidence of MBs in an inception cohort of real life NVAf patients anticoagulated with VKAs and monitored in ACC. Though in the Register the number and length of follow-up available for DOAC-treated patients is still low and insufficient for sound conclusions it seems that MB occurrence in these patients is similar to that in well monitored VKA-treated patients

Disclosure of Interest: None declared.

PO676-WED

Eahad-DB: a combined coagulation factor variant databases resource for the clinical and scientific communities

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Background: Locus-specific databases available for coagulation factors (CoagDB) are a highly valued clinical/scientific resource; providing searchable lists of sequence variants and information on the phenotype-genotype correlations in patients with inherited bleeding disorders. For several coagulation factors multiple CoagDB exist, each containing different (and sometimes conflicting) information causing confusion amongst users. CoagDB also receive little or no administrative/financial support, putting their long-term viability at risk.

Aims: To create a combined CoagDB portal and sophisticated new searchable websites for each factor to provide centralised administrative/financial support to CoagDB curators and consistent information to CoagDB users.

Methods: Initially focusing on CoagDB for von Willebrand factor (*VWF*) and coagulation factors IX (*F9*), VIII (*F8*), VII (*F7*) and XI (*F11*) the European Association for Haemophilia and Allied Disorders (EAHAD) evaluated current information, data submission and data curation processes and addressed data protection and associated ethical issues.

Results: Access to all five CoagDB has been combined under a central EAHAD portal (www.eahad-db.org) allowing a common data protection policy and variant submission process to be adopted. Information on known variants and associated phenotypic data is provided for all factors, while enhanced databases (currently available for *F8* and *F9*) provide additional information, e.g. regarding protein structure and sequence alignments.

Conclusion: EAHAD have established a common CoagDB front-end, initially incorporating *F7*, *F8*, *F9*, *F11* and *VWF* with plans for incorporation of further genes. This portal will serve as a worldwide repository providing accurate and consistent information for all coagulation factors relevant to all those working in the clinical and scientific sectors, while also ensuring long-term administrative/financial support.

Disclosure of Interest: None declared.

PO677-WED

Polyhedrocytes in whole blood clots of patients with venous thromboembolism taking vitamin k antagonists: a role of the quality of anticoagulation

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Background: Polyhedrocytes are formed *in vivo* and able to form impermeable structures within contracted blood clots crucial for hemostasis (Cines DG et al. *Blood* 2014). Factors that determine their occurrence in venous thromboembolism (VTE) are unknown.

Aims: We hypothesized that anticoagulant treatment can alter the pattern of polyhedrocyte formation.

Methods: We analyzed whole venous blood clots formed after 24 h at 22 °C by adding 1 U mL⁻¹ human thrombin and 5 mM calcium chloride to citrated blood samples obtained from 20 VTE patients on long-term vitamin K antagonist (VKA) therapy. They had a median time in the therapeutic range (TTR) of 70% (range 40–90%) and international normalized ratio (INR) of 2.23. The reference group represented healthy volunteers. Clots were fixed in 2.5% glutaraldehyde and prepared for scanning electron microscopy. We calculated the area covered by various forms of erythrocytes and the contraction ratio defined as a difference between the area of native red blood cells (RBCs) and polyhedral erythrocytes within the clot.

Results: In VTE patients taking VKA polyhedrocytes were observed in 19 (95%) clots. The contraction ratio was 43%. The mean clot area covered by polyhedrocytes was 14% in VTE patients and 40% in healthy controls ($P < 0.05$). The area of the clot covered by polyhedrocytes $> 10\%$ ($n = 14$) was found in VTE patients with higher C-reactive protein, triglycerides, and RBC count (all $P < 0.05$) but not with elevated fibrinogen. Of note, the area of the clot covered by polyhedrocytes correlated inversely with the INR and TTR ($r = -0.40$ and $r = -0.68$, respectively; both $P < 0.05$). The contraction ratio was inversely associated with the RBCs distribution width and INR ($r = -0.62$ and $r = -0.66$, respectively; both $P < 0.05$).

Conclusion: Anticoagulation in VTE patients diminishes the ability to form polyhedrocytes within blood clots in association with quality of the therapy. Impaired polyhedrocyte formation in patients on VKA might be a novel mechanism enhancing removal of intravascular thrombi.

Disclosure of Interest: None declared.